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(54) Title: ASPERGILLUS OCHRACEUS 11 ALPHA HYDROXYLASE AND OXIDOREDUCTASE

(57) Abstract: The present invention relates to a novel cytochrome P450-like enzyme (*Aspergillus ochraceus*) isolated from cDNA library generated from the mRNA of *Aspergillus ochraceus* spores. When the cDNA encoding the 11 alpha hydroxylase was co-expressed in *Spodoptera frugiperda* (Sf 9) insect cells with the cDNA encoding human oxidoreductase as an electron donor, it successfully catalyzed the conversion of the steroid substrate 4-androstene-3,17-dione (AD) to 11 alpha-hydroxy-AD as determined by HPLC analysis. The invention also relates to nucleic acid molecules associated with or derived from these cDNAs including complements, homologues and fragments thereof, and methods of using these nucleic acid molecules, to generate, for example, polypeptides and fragments thereof. The invention also relates to the generation of antibodies that recognizes the *A. ochraceus* 11 alpha hydroxylase and oxidoreductase and methods of using these antibodies to detect the presence of these native and recombinant polypeptides within unmodified and transformed host cells, respectively. The invention also provides methods of expressing the *Aspergillus* 11 alpha hydroxylase gene separately, or in combination with human or *Aspergillus* oxidoreductase, in heterologous host cells, to facilitate the bioconversion of steroid substances to their 11 alpha hydroxy-counterparts.

***Aspergillus ochraceus* 11 alpha hydroxylase and oxidoreductase**

Priority

5 The present application claims priority under Title 35, United States Code,
§ 119 of United States Provisional Application Serial No. 60/244,300, filed October
30, 2000.

Field of the invention

10 The present invention relates to a novel cytochrome P450-like enzyme
(*Aspergillus ochraceus* 11 alpha hydroxylase) and an oxidoreductase (*Aspergillus*
ochraceus oxidoreductase) isolated from cDNA library generated from the mRNA of
Aspergillus ochraceus spores. When the cDNA encoding the 11 alpha hydroxylase
was co-expressed in *Spodoptera frugiperda* (Sf-9) insect cells with the cDNA
encoding human oxidoreductase as an electron donor, it successfully catalyzed the
conversion of the steroid substrate 4-androstene-3,17-dione (AD) to 11 alpha-
15 hydroxy-AD as determined by HPLC analysis. The invention also relates to nucleic
acid molecules associated with or derived from these cDNAs including
complements, homologues and fragments thereof, and methods of using these
nucleic acid molecules, to generate, for example, polypeptides and fragments
thereof. The invention also relates to the generation of antibodies that recognize
20 the *A. ochraceus* 11 alpha hydroxylase and oxidoreductase and methods of using
these antibodies to detect the presence of these native and recombinant
polypeptides within unmodified and transformed host cells, respectively. The
invention also provides methods of expressing the *Aspergillus* 11 alpha
hydroxylase gene separately, or in combination with human or *Aspergillus*
25 oxidoreductase, in heterologous host cells, to facilitate the bioconversion of steroid
substrates to their 11 alpha hydroxy-counterparts.

Background of the invention

30 Microbial transformation or bioconversion reactions have long been used to
facilitate the chemical synthesis of a wide variety of pharmaceutical products.
Stereospecific reactions carried out under mild enzymatic conditions frequently
offer advantages over comparable chemical processes which result in undesirable

side products. Microorganisms also have the ability to carry out simultaneous independent or sequential reactions on a substrate molecule, minimizing the number of distinct steps in a synthesis and reducing the total cost of the desired intermediate or end product.

5 General features of microbial systems used as biocatalysts for the transformation of organic compounds has been reviewed (See e.g., Goodhue, Charles T., *Microb. Transform. Bioact. Compd.*, 1: 9-44, 1982). Biotransformations can be carried out, for example, in continuous cultures or in batch cultures. Enzymes secreted from the microorganism react with a substrate, and the product
10 can be recovered from the medium. Intracellular enzymes can also react with a substrate if it is able to enter cells by an active or a passive diffusion process. Immobilized, dried, permeabilized, and resting cells, and spores have also been used for microbial transformations. The use of cell extracts and purified enzymes in solution, or immobilized on carriers, may eventually offer significant cost or
15 control advantages over traditional fermentation methods.

 Bioconversion reactions have been widely used in the field of steroids (Kieslich, K.; Sebek, O. K. *Annu. Rep. Ferment. Processes* 3: 275-304, 1979; Kieslich, Klaus. *Econ. Microbiol.*, 5 (Microb. Enzymes Bioconvers.): 369-465, 1980). A variety of reactions have been characterized, including hydroxylation,
20 epoxidation, oxidation, dehydrogenation, ring and side chain degradation, reduction, hydrolysis, and isomerization reactions. Many types of microorganisms have also been used including species as diverse, for example, as *Acremonium*, *Aspergillus*, *Rhizopus*, *Fusarium*, *Penicillium*, *Streptomyces*, *Actinomyces*, *Nocardia*, *Pseudomonas*, *Mycobacterium*, *Arthrobacter* and *Bacillus*.

25 A variety of approaches have been used to facilitate the hydroxylation of intermediates used in the synthesis of commercially-important steroid compounds. US patent 4,588,683, for example, describes a method of preparing 11 beta, 17 alpha, 20, 21 tetrahydroxy steroids by incubating substrate compounds in a medium comprising a fungal culture of the genus *Curvularia* capable of effecting
30 11 beta hydroxylation. *Aspergillus ochraceus* cultures and preparations of mycelia have also been used to convert progesterone and other steroids to their corresponding 11 alpha hydroxy forms (Tan, L. and Falardeau, P., 1970; Tan L., and Falardeau P., *J. Steroid Biochem.* 1: 221-227, 1970; Samanta, T.B. et al., *Biochem. J.* 176, 593-594, 1978; Jayanthi, C.R. et al., *Biochem. Biophys. Res. Commun.* 106: 1262-1268, 1982).
35

The advent of new and expanded clinical uses of steroids for the treatment of a wide variety of disorders has created a need for improved methods for the production of steroid compounds and their intermediates on a commercial scale. U.S. patent 4,559,332, for example, describes a number of methods for the preparation of 20-spiroxane series of steroid compounds, including methods for the preparation of eplerenone methyl hydrogen 9,11 α -epoxy-17 α -hydroxy-3-oxopregn-4-ene-7 α ,21-dicarboxylate, γ -lactone (also referred to as eplerenone or epoxymexrenone) and related compounds. WO 98/25948 and U.S. application 09/319,673 describe novel processes for the preparation of 9,11-epoxy steroid compounds, especially those of the 20-spiroxane series and their analogs, novel intermediates useful in the preparation of steroid compounds, and processes for the preparation of such novel intermediates. United States Patent 6,046,023 discloses improved methods for the microbial transformation of canrenone or estr-4-ene-3,17-dione into its 11 α -hydroxy analogue using microorganisms of the genus *Aspergillus*, *Rhizopus*, and *Pestalotia*, using steroid substrates having a purity of less than 97% and more than 90% at a concentration greater than 10 g/L.

Many modern, systematic approaches needed to optimize bioconversion of particular steroid intermediates are often hindered by insufficient biochemical knowledge of the enzymes involved in their synthesis and degradation. Eukaryotic cytochromes P450 appear to be associated with the endoplasmic reticulum (ER) or mitochondrial membranes. The electron donor for ER-associated cytochrome P450 enzymes is often an FAD/FMN-dependent NADPH-cytochrome P450 oxidoreductase. Electron transfer in the mitochondrial cytochromes P450 is usually mediated by an NADPH-ferredoxin oxidoreductase and ferredoxin. The specific electron donors known to be involved in mammalian steroidogenesis, are also called adrenodoxin reductase and adrenodoxin, respectively.

While fungal biotransformations are known to be mediated by cytochrome P450 enzymes, many of these enzymes are extremely difficult to purify in an enzymatically-active form (van den Brink et al., *Fungal Genetics and Biology* 23, 1-17, 1998). Many fungal P450 enzymes appear to be associated with the endoplasmic reticulum (van den Brink et al., *Fungal Genetics and Biology* 23, 1-17, 1998). Yeast have an adrenodoxin reductase homologue which was shown to couple with a mammalian 11 beta hydroxylase *in vitro*. (Lacour et al., *Journal of Biological Chemistry* 273, 23984-23992, 1998). In contrast, the electron donor which couples with *Aspergillus ochraceus* 11 alpha hydroxylase was predicted to be an NADPH-cytochrome P450 oxidoreductase (Samanta and Ghosh, *J Steroid Biochem* 28, 327-32, 1987). The steroid 11 alpha hydroxylation complex in

Rhizopus nigricans also appears to require an NADPH-cytochrome p450 oxidoreductase (Makovec and Breskvar, *Arch Biochem Biophys.* **357**, 310-6, 1998). Amplification of cytochrome *R. nigricans* P450 and NADPH-cytochrome P450 reductase activities in preparations of progesterone-induced fungal mycelia may
5 the facilitate biochemical characterization of both enzymes (Makovec and Breskvar, *Pflugers Arch - Eur J. Physiol* **439**(Suppl): R111-R112, 2000).

Aspergillus ochraceus spores have been shown to catalyze the 11 alpha hydroxylation of steroid substrates such as progesterone (Dutta TK, Datta J, Samanta TB, *Biochem. Biophys. Res. Commun.* **192**:119-123, 1993). *A. fumigatus*
10 is also known to exhibit a steroid 11 alpha hydroxylase activity (Smith et al., *J Steroid Biochem Mol Biol* **49**: 93-100, 1994). The *A. fumigatus* enzyme is distinguished from the *A. ochraceus* enzyme, in that it appears to be a cytochrome P450 with dual site-specificity for 11 alpha and 15 beta hydroxylation and, unlike the *A. ochraceus* hydroxylase, appears to be non-inducible.

15 Despite recent advances in sequencing technologies, detailed knowledge about the structural relationships of fungal cytochrome P450s gleaned from nucleotide sequence data remains primitive. Breskvar *et al.*, (*Biochem. Biophys. Res. Commun* 1991; **178**, 1078-1083, 1991) have described a genomic DNA sequence from *Rhizopus nigricans* for a putative P-450 encoding an
20 11 α -hydroxylase for progesterone. This sequence may not be complete, however, since the predicted amino acid sequence lacks the canonical heme-binding motif, FxxGxxxCxG, which is common to almost all known cytochrome P-450 enzymes. (Nelson *et al*, *Pharmacogenetics* **6**: 1-42, 1996).

The cloning and characterization of the NADPH cytochrome P450 oxidoreductase (cprA) gene of *Aspergillus niger* has been described (van den Brink,
25 J., et al., Genbank accession numbers Z26938, CAA81550, 1993, unpublished). The primary structure of *Saccharomyces cerevisiae* NADPH-cytochrome P450 reductase has also been deduced from the nucleotide sequence of its cloned gene (Yabusaki et al., *J. Biochem.* **103**(6): 1004-1010, 1988).

30 Several other approaches have been used to facilitate the cloning and analysis of steroid enzymes. U.S. patents 5,422,262, 5,679,521, and European patent EP 0 528 906 B1, for example, describes the expression cloning of steroid 5 alpha reductase, type 2. U.S. patent 5,869,283, for example, describes an expression cassette comprising heterologous DNAs encoding two or more enzymes,
35 each catalyzing an oxidation step involved conversion of cholesterol into

hydrocortisone, including the conversion of cholesterol to pregnenolone; the conversion of pregnenolone to progesterone; the conversion of progesterone to 17 α -hydroxy-progesterone; the conversion of 17 α -hydroxyprogesterone to cortexolone; and the conversion of cortexolone to hydrocortisone.

5 The sequences of *Aspergillus ochraceus* 11 alpha hydroxylase and *A. ochraceus* oxidoreductase have not been reported. Knowledge about their sequence could greatly facilitate the development of expression vectors and recombinant host strains that can carry out more efficient bioconversions of steroid intermediates and the synthesis of end products on a commercial scale without the problems
10 associated with partially-characterized host strains or an incomplete understanding of the enzymes involved in steroidogenesis. The present invention overcomes many of the limitations discussed above by identifying enzymes capable of carrying out the 11 alpha hydroxylation of steroids. This approach not only greatly facilitates the use of 11 alpha hydroxylation, but also permits the
15 development of new strategies for the identification of similar enzymes from other fungi, the cloning of other enzymes involved in steroidogenesis from *Aspergillus ochraceus* and other microorganisms, and the development of improved host strains or methods using free cells or immobilized cells or enzymes in bioconversion reactions. Similar approaches could also be developed to aid in the construction of
20 expression vectors and recombinant host strains that are more amenable to propagation and control than wild-type microorganisms now commonly used for bioconversion in large scale bioreactors.

Summary of the invention

In its broadest scope, the present invention provides a method to clone
25 enzymes involved in steroid metabolism and use of these enzymes to produce novel steroid intermediates and end-products. One aspect of the claimed invention is to provide a novel enzyme 11 alpha hydroxylase and oxidoreductase, and their nucleic acids, proteins, peptides, fragments, and homologues. The invention also relates to methods of identifying and cloning other enzymes involved in steroid metabolism.
30 The invention also covers novel vectors and host cells, a novel method for making heterologous proteins by using the above vectors, and a method for identifying the substrate specificity of the cloned enzymes.

The invention provides a means for determining the substrate specificity of the cloned 11 alpha hydroxylase, allelic variants, muteins, and fusion proteins
35 thereof, permitting evaluation of a broad array of steroid substrates including 3

keto delta 4,5 steroids (3 keto delta 4 steroids); 3 keto delta 4, 5 delta 6, 7 steroids (3 keto delta 4 delta 6 steroids); 3 keto delta 6, 7 steroids (3 keto delta 6 steroids); or 3 keto delta 1, 2 delta 4, 5 steroids (3 keto delta 1 delta 4 steroids). Preferred substrates for testing include (a) canrenone; (b) androstenedione; (c) aldona; (d) ADD (1,4 androstenedienedione) (e) mexrenone; (f) 6 beta mexrenone; (g) 9 alpha mexrenone; (h) 12 beta mexrenone; (i) delta 12 mexrenone; (j) testosterone; (k) progesterone; (l) mexrenone 6,7-bis-lactone; and (m) mexrenone 7,9-bis-lactone. Preferably the cloned 11 alpha hydroxylase, allelic variants, muteins, and fusion proteins thereof do not also catalyze a second hydroxylation selected from the group consisting of 15 alpha or beta hydroxylation, 6 alpha or beta hydroxylation, 7 alpha or beta hydroxylation, 9 alpha or beta hydroxylation, 12 alpha or beta hydroxylation, and 17 alpha or beta hydroxylation of substrates selected from the group consisting of 3 keto delta 4,5 steroids; 3 keto delta 4, 5 delta 6, 7 steroids; or 3 keto delta 6, 7 steroids. Most preferably the cloned 11 alpha hydroxylase, allelic variants, muteins, and fusion proteins thereof do not catalyze the 15 beta hydroxylation of substrates selected from the group consisting of 3 keto delta 4,5 steroids; 3 keto delta 4, 5 delta 6, 7 steroids; or 3 keto delta 6, 7 steroids.

The invention provides an isolated and purified nucleic acid, encoding *Aspergillus ochraceus* 11 alpha hydroxylase. It also provides an isolated DNA, cDNA, gene, and an allele of the gene encoding *Aspergillus ochraceus* 11 alpha hydroxylase. Preferably the isolated and purified nucleic acid is as set forth in SEQ ID NO: 01. Preferably the isolated DNA, cDNA, gene, and an allele of the gene is as set forth in SEQ ID NO: 01.

The invention provides an isolated protein having the amino acid sequence of *Aspergillus ochraceus* 11 alpha hydroxylase. It also provides an isolated variant of *Aspergillus ochraceus* 11 alpha hydroxylase, and a fusion protein comprising this hydroxylase. Preferably the protein is as set forth in SEQ ID NO: 2. It also provides for variant of the protein set forth in SEQ ID NO: 2.; a polypeptide which comprises SEQ ID NO: 2 with at least one conservative amino acid substitution; polypeptides, with an amino acid sequence at least 99%, 95%, 90%, 75%, and 50% identical to SEQ ID NO: 2.

The invention provides an isolated and purified nucleic acid, encoding *Aspergillus ochraceus* 11 alpha oxidoreductase. It also provides an isolated DNA, cDNA, gene, and allele of the gene encoding *Aspergillus ochraceus* oxidoreductase. Preferably, the isolated and purified nucleic acid, wherein said nucleic acid

sequence is as set forth in SEQ ID NO: 5. It also provides for an isolated DNA, cDNA, gene, and allele of the gene set forth in SEQ ID NO: 5.

The invention provides an isolated protein having the amino acid sequence of *Aspergillus ochraceus* oxidoreductase. It also provides an isolated variant of the protein having the amino acid sequence of *Aspergillus ochraceus* oxidoreductase, and a fusion protein comprising the amino acid sequence of *Aspergillus ochraceus* oxidoreductase. Preferably the isolated protein has the amino acid sequence set forth in SEQ ID NO: 6. It also provides an isolated variant of a protein set forth in SEQ ID NO: 6, a purified polypeptide, the amino acid sequence of which comprises SEQ ID NO: 6 with at least one conservative amino acid substitution; and a polypeptides with an amino acid sequence at least 99%, 95%, 90%, 75%, and 50% identical to SEQ ID NO: 6.

The invention provides an isolated and purified nucleic acid encoding an enzyme that can catalyze the 11 alpha hydroxylation of 3 keto delta 4,5 steroids (3 keto delta 4 steroids); 3 keto delta 4, 5 delta 6, 7 steroids (3 keto delta 4 delta 6 steroids); 3 keto delta 6, 7 steroids (3 keto delta 6 steroids); or 3 keto delta 1, 2 delta 4, 5 steroids (3 keto delta 1 delta 4 steroids). Preferably the enzyme does not catalyze the 15 beta hydroxylation of 3 keto delta 4,5 steroids; 3 keto delta 4, 5 delta 6, 7 steroids; or 3 keto delta 6, 7 steroids. More preferably, the hydroxylation is selected from the group consisting of: (a) canrenone to 11 alpha hydroxy canrenone; (b) androstenedione to 11 alpha hydroxy androstenedione; (c) aldona to 11 alpha hydroxy aldona; (d) ADD (1,4 androstenedienedione) to 11 alpha hydroxy ADD; (e) mexrenone to 11 alpha hydroxy mexrenone; (f) 6 beta mexrenone to 11 alpha hydroxy 6 beta mexrenone; (g) 9 alpha mexrenone to 11 alpha hydroxy 9 alpha mexrenone; (h) 12 beta mexrenone to 11 alpha hydroxy 12 beta mexrenone; (i) delta 12 mexrenone to 11 alpha hydroxy delta 12 mexrenone; (j) testosterone to 11 alpha hydroxy testosterone; (k) progesterone to 11 alpha hydroxy progesterone; (l) mexrenone 6,7-bis-lactone to 11 alpha hydroxy mexrenone 6,7-bis-lactone; and (m) mexrenone 7,9-bis-lactone to 11 alpha hydroxy mexrenone 7,9-bis-lactone. More preferably, the hydroxylation is selected from the group consisting of: (a) canrenone to 11 alpha hydroxy canrenone; (b) androstenedione to 11 alpha hydroxy androstenedione; (c) aldona to 11 alpha hydroxy aldona; and (d) ADD (1,4 androstenedienedione) to 11 alpha hydroxy ADD. Most preferably the hydroxylation is from canrenone to 11 alpha hydroxy canrenone.

The invention also provides a method of expressing a protein that can catalyze the 11 alpha hydroxylation of 3 keto delta 4,5 steroids; 3 keto delta 4, 5

delta 6, 7 steroids; 3 keto delta 6, 7 steroids; or 3 keto delta 1, 2 delta 4, 5 steroids comprising; (a) transforming or transfecting host cells with an expression cassette comprising a promoter operably linked to a nucleic acid that encodes said protein, and (b) expressing said protein in said host cells. The invention also provides for a method of producing the protein further comprising the step of recovering said protein. Preferably, this protein is *Aspergillus ochraceus* 11 alpha hydroxylase. More preferably, this method further comprises expressing an electron donor protein, wherein said electron donor protein can donate electrons to said protein that can catalyze the 11 alpha hydroxylation of 3 keto delta 4,5 steroids; 3 keto delta 4, 5 delta 6, 7 steroids; 3 keto delta 6, 7 steroids; or 3 keto delta 1, 2 delta 4, 5 steroids. Preferably, the electron donor protein is selected from the group consisting of human oxidoreductase and *Aspergillus ochraceus* oxidoreductase. More preferably the electron donor protein is *Aspergillus ochraceus* oxidoreductase. More preferably, the nucleic acid encoding said steroid 11 alpha hydroxylase and said electron donor protein are on separate expression cassettes. More preferably, the nucleic acid encoding said steroid 11 alpha hydroxylase and said electron donor protein are on the same expression cassettes. Even more preferably, the steroid 11 alpha hydroxylase is *Aspergillus ochraceus* 11 alpha hydroxylase and said electron donor protein is human oxidoreductase. Even more preferably, the steroid 11 alpha hydroxylase is *Aspergillus ochraceus* 11 alpha hydroxylase and said electron donor protein is *Aspergillus ochraceus* oxidoreductase. Preferably, the expression cassette is on an expression vector. More preferably, the expression vector is a baculovirus. Even more preferably, the baculovirus is a nuclear polyhedrosis virus is selected from the group consisting of *Autographa californica* nuclear polyhedrosis virus and *Bombyx mori* nuclear polyhedrosis virus. Most preferably, the nuclear polyhedrosis virus is *Autographa californica* nuclear polyhedrosis virus. Preferably, the host cells are insect cells. More preferably, the insect cells are selected from the group consisting of *Spodoptera frugiperda*, *Trichoplusia ni*, *Autographa californica*, and *Manduca sexta* cells. Most preferably the insect cells are *Spodoptera frugiperda* cells. The invention also provides a for a method of expressing a protein wherein the *Aspergillus ochraceus* 11 alpha hydroxylase is SEQ ID NO: 2; the human oxidoreductase is SEQ ID NO: 4; and the *Aspergillus ochraceus* oxidoreductase is SEQ ID NO: 6.

The invention also provides for an isolated and purified polypeptide that can catalyze the 11 alpha hydroxylation of 3 keto delta 4,5 steroids (3 keto delta 4 steroids); 3 keto delta 4, 5 delta 6, 7 steroids (3 keto delta 4 delta 6 steroids); 3 keto delta 6, 7 steroids (3 keto delta 6 steroids); or 3 keto delta 1, 2 delta 4, 5 steroids (3

keto delta 1 delta 4 steroids). Preferably, the polypeptide does not catalyze the 15
beta hydroxylation of 3 keto delta 4,5 steroids; 3 keto delta 4, 5 delta 6, 7 steroids;
or 3 keto delta 6, 7 steroids. More preferably, the hydroxylation is selected from
the group consisting of: (a) canrenone to 11 alpha hydroxy canrenone; (b)
5 androstenedione to 11 alpha hydroxy androstenedione; (c) aldona to 11 alpha
hydroxy aldona; (d) ADD (1,4 androstenedienedione) to 11 alpha hydroxy ADD; (e)
mexrenone to 11 alpha hydroxy mexrenone; (f) 6 beta mexrenone to 11 alpha
hydroxy 6 beta mexrenone; (g) 9 alpha mexrenone to 11 alpha hydroxy 9 alpha
mexrenone; (h) 12 beta mexrenone to 11 alpha hydroxy 12 beta mexrenone; (i)
10 delta 12 mexrenone to 11 alpha hydroxy delta 12 mexrenone; (j) testosterone to 11
alpha hydroxy testosterone; (k) progesterone to 11 alpha hydroxy progesterone; (l)
mexrenone 6,7-bis-lactone to 11 alpha hydroxy mexrenone 6,7-bis-lactone; and (m)
mexrenone 7,9-bislactone to 11 alpha hydroxy mexrenone 7,9-bislactone. More
preferably, the hydroxylation is selected from the group consisting of: (a)
15 canrenone to 11 alpha hydroxy canrenone; (b) androstenedione to 11 alpha hydroxy
androstenedione; (c) aldona to 11 alpha hydroxy aldona; and (d) ADD (1,4
androstenedienedione) to 11 alpha hydroxy ADD. Most preferably the
hydroxylation is from canrenone to 11 alpha hydroxy canrenone.

The invention also provides for an expression cassette comprising a
20 promoter operably linked to an isolated and purified nucleic acid encoding a
polypeptide that can catalyze the 11 alpha hydroxylation of 3 keto delta 4,5
steroids (3 keto delta 4 steroids); 3 keto delta 4, 5 delta 6, 7 steroids (3 keto delta 4
delta 6 steroids); 3 keto delta 6, 7 steroids (3 keto delta 6 steroids); or 3 keto delta
1, 2 delta 4, 5 steroids (3 keto delta 1 delta 4 steroids). More preferably, the
25 hydroxylation is selected from the group consisting of: (a) canrenone to 11 alpha
hydroxy canrenone; (b) androstenedione to 11 alpha hydroxy androstenedione; (c)
aldona to 11 alpha hydroxy aldona; (d) ADD (1,4 androstenedienedione) to 11
alpha hydroxy ADD; (e) mexrenone to 11 alpha hydroxy mexrenone; (f) 6 beta
mexrenone to 11 alpha hydroxy 6 beta mexrenone; (g) 9 alpha mexrenone to 11
30 alpha hydroxy 9 alpha mexrenone; (h) 12 beta mexrenone to 11 alpha hydroxy 12
beta mexrenone; (i) delta 12 mexrenone to 11 alpha hydroxy delta 12 mexrenone;
(j) testosterone to 11 alpha hydroxy testosterone; (k) progesterone to 11 alpha
hydroxy progesterone; (l) mexrenone 6,7-bis-lactone to 11 alpha hydroxy
mexrenone 6,7-bis-lactone; and (m) mexrenone 7,9-bislactone to 11 alpha hydroxy
35 mexrenone 7,9-bislactone. More preferably, the hydroxylation is selected from the
group consisting of: (a) canrenone to 11 alpha hydroxy canrenone; (b)
androstenedione to 11 alpha hydroxy androstenedione; (c) aldona to 11 alpha

hydroxy aldona; and (d) ADD (1,4 androstenedienedione) to 11 alpha hydroxy ADD. Most preferably the hydroxylation is from canrenone to 11 alpha hydroxy canrenone.

5 The invention also provides for an expression cassette comprising a promoter operably linked to an isolated and purified nucleic acid encoding *Aspergillus ochraceus* oxidoreductase. Preferably the nucleic acid is SEQ ID NO: 6.

The invention also provides for an expression cassette comprising a heterologous DNA encoding an enzyme from the metabolic pathway for the synthesis of sitosterol to eplerenone wherein said enzyme catalyzes at least one
10 conversion selected from the group consisting of: (a) canrenone to 11 alpha hydroxy canrenone; (b) androstenedione to 11 alpha hydroxy androstenedione; (c) aldona to 11 alpha hydroxy aldona; (d) ADD (1,4 androstenedienedione) to 11 alpha hydroxy ADD; (e) mexrenone to 11 alpha hydroxy mexrenone; (f) 6 beta mexrenone to 11 alpha hydroxy 6 beta mexrenone; (g) 9 alpha mexrenone to 11 alpha hydroxy 9
15 alpha mexrenone; (h) 12 beta mexrenone to 11 alpha hydroxy 12 beta mexrenone; (i) delta 12 mexrenone to 11 alpha hydroxy delta 12 mexrenone; (j) testosterone to 11 alpha hydroxy testosterone; and (k) progesterone to 11 alpha hydroxy progesterone; (l) mexrenone 6,7-bis-lactone to 11 alpha hydroxy mexrenone 6,7-bis-lactone; and (m) mexrenone 7,9-bis-lactone to 11 alpha hydroxy mexrenone 7,9-
20 bislactone and wherein the heterologous DNA is operably linked to control sequences required to express the encoded enzymes in a recombinant host. Preferably the heterologous DNA coding sequences in the expression cassette are selected from the group consisting of the following genus and species: *Aspergillus ochraceus*, *Aspergillus ochraceus*, *Aspergillus niger*, *Aspergillus nidulans*, *Rhizopus oryzae*, *Rhizopus stolonifer*, *Streptomyces fradiae*, *Bacillus megaterium*, *Pseudomonas cruciviae*, *Trichothecium roseum*, *Fusarium oxysporum* *Rhizopus arrhizus*, *Absidia coerula*, *Absidia glauca*, *Actinomucor elegans*, *Aspergillus flavipes*, *Aspergillus fumigatus*, *Beauveria bassiana*, *Botryosphaeria obtusa*, *Calonectria decora*, *Chaetomium cochliodes*, *Corynespora cassiicola*,
25 *Cunninghamella blakesleeana*, *Cunninghamella echinulata*, *Cunninghamella elegans*, *Curvularia clavata*, *Curvularia lunata*, *Cylindrocarpon radiculicola*, *Epicoccum humicola*, *Gongronella butleri*, *Hypomyces chrysospermus*, *Monosporium olivaceum*, *Mortierella isabellina*, *Mucor mucedo*, *Mucor griseocyanus*, *Myrothecium verrucaria*, *Nocardia corallina*, *Paecilomyces carneus*,
30 *Penicillium patulum*, *Pithomyces atroolivaceus*, *Pithomyces cynodontis*, *Pycnosporium* sp., *Saccharopolyspora erythrae*, *Sepedonium chrysospermum*, *Stachylidium bicolor*, *Streptomyces hygroscopicus*, *Streptomyces purpurascens*,

Syncephalastrum racemosum, *Thamnostylum piriforme*, *Thielavia terricola*, and *Verticillium theobromae*, *Cephalosporium aphidicola*, *Cochliobolus lunatas*, *Tieghemella orchidis*, *Tieghemella hyalospora*, *Monosporium olivaceum*, *Aspergillus ustus*, *Fusarium graminearum*, *Verticillium glaucum*, and *Rhizopus nigricans*. More preferably, the genus and species are selected from the group consisting of *Aspergillus ochraceus*, *Aspergillus ochraceus*, *Aspergillus niger*, *Aspergillus nidulans*, *Rhizopus oryzae*, *Rhizopus stolonifer*, *Streptomyces fradiae*, *Bacillus megaterium*, *Pseudomonas cruciviae*, *Trichothecium roseum*, *Fusarium oxysporum*, *Rhizopus arrhizus*, and *Monosporium olivaceum*. Most preferably, genus and species is *Aspergillus ochraceus*.

Preferably, the recombinant host cell and progeny thereof comprise at least one expression cassette. More preferably, the host is a microorganism. Most preferably, the host is a bacterium. The invention also provides for a process for making one or more enzymes from the metabolic pathway for the transformation of sitosterol to eplerenone comprising incubating the recombinant host cell in a nutrient medium under conditions where the one or more enzymes encoded by the heterologous DNA are expressed and accumulate. More preferably the process comprises the steps of: (a) incubating the compound to be oxidized in the presence the recombinant host cells under conditions where the compound is hydroxylated and the hydroxylated product accumulates, and (b) recovering the hydroxylated product. Most preferably, the process comprises the steps of: (a) incubating the compound to be oxidized in the presence of the enzymes produced under conditions where the compound is hydroxylated and the hydroxylated product accumulates, and (b) recovering the hydroxylated product. The invention also provides for a host cells harboring an expression cassette. More preferably the expression cassette is integrated into the chromosome of said host cell. More preferably, the expression cassette is integrated into an expression vector.

The invention also provides for a method of determining the specific activity of a cloned 11 alpha hydroxylase comprising the steps of; (a) transforming host cells with an expression vector comprising a nucleic acid that encodes said 11 alpha hydroxylase, (b) expressing said 11 alpha hydroxylase in said host cells; (c) preparing subcellular membrane fractions from said cells, (d) incubating said subcellular membrane fractions with a steroid substrate, and (e) monitoring conversion of the steroid substrate to its 11 alpha hydroxy steroid counterpart. Preferably, the further comprises transforming host cells with an expression vector nucleic acid that encodes an oxidoreductase, and expressing said oxidoreductase in said host cells. More preferably, the oxidoreductase is human or *Aspergillus*

ochraceus. Most preferably the oxidoreductase is human oxidoreductase. Most preferably the oxidoreductase is *Aspergillus ochraceus* oxidoreductase.

The invention also provides for a protein having SEQ ID NO: 2 and variants thereof that are at least 95% identical to SEQ ID NO: 2 and catalyze the
5 11 alpha hydroxylation of 3 keto delta 4,5 steroids; 3 keto delta 4, 5 delta 6, 7 steroids; 3 keto delta 6, 7 steroids; or 3 keto delta 1, 2 delta 4, 5 steroids, wherein said hydroxylation is selected from the group consisting of: (a) canrenone to 11 alpha hydroxy canrenone; (b) androstenedione to 11 alpha hydroxy androstenedione; (c) aldona to 11 alpha hydroxy aldona; (d) ADD (1,4
10 androstenedienedione) to 11 alpha hydroxy ADD; (e) mexrenone to 11 alpha hydroxy mexrenone; (f) 6 beta mexrenone to 11 alpha hydroxy 6 beta mexrenone; (g) 9 alpha mexrenone to 11 alpha hydroxy 9 alpha mexrenone; (h) 12 beta mexrenone to 11 alpha hydroxy 12 beta mexrenone; (i) delta 12 mexrenone to 11 alpha hydroxy delta 12 mexrenone; (j) testosterone to 11 alpha hydroxy testosterone; and (k) progesterone to 11 alpha hydroxy progesterone. Preferably
15 the enzyme does not catalyze the 15 beta hydroxylation of 3 keto delta 4,5 steroids; 3 keto delta 4, 5 delta 6, 7 steroids; or 3 keto delta 6, 7 steroids.

The invention provides an isolated and purified nucleic acid encoding an enzyme that can catalyze the 11 alpha hydroxylation of 3 keto delta 4,5 steroids (3
20 keto delta 4 steroids); 3 keto delta 4, 5 delta 6, 7 steroids (3 keto delta 4 delta 6 steroids); 3 keto delta 6, 7 steroids (3 keto delta 6 steroids); or 3 keto delta 1, 2 delta 4, 5 steroids (3 keto delta 1 delta 4 steroids) wherein the hydroxylation is selected from the group consisting of: (a) canrenone to 11 alpha hydroxy canrenone; (b) androstenedione to 11 alpha hydroxy androstenedione; (c) aldona to 11 alpha
25 hydroxy aldona; (d) ADD (1,4 androstenedienedione) to 11 alpha hydroxy ADD; (e) mexrenone to 11 alpha hydroxy mexrenone; (f) 6 beta mexrenone to 11 alpha hydroxy 6 beta mexrenone; (g) 9 alpha mexrenone to 11 alpha hydroxy 9 alpha mexrenone; (h) 12 beta mexrenone to 11 alpha hydroxy 12 beta mexrenone; (i) delta 12 mexrenone to 11 alpha hydroxy delta 12 mexrenone; (j) testosterone to 11
30 alpha hydroxy testosterone; and (k) progesterone to 11 alpha hydroxy progesterone. Preferably the enzyme does not catalyze the 15 beta hydroxylation of 3 keto delta 4,5 steroids; 3 keto delta 4, 5 delta 6, 7 steroids; or 3 keto delta 6, 7 steroids.

The invention also provides for a purified polypeptide, the amino acid
35 sequence of which is selected from the group consisting of SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25.

The invention provides for a purified immunogenic polypeptide, the amino acid sequence of which comprises at least ten consecutive residues of SEQ ID NO: 2.

5 The invention provides for an isolated and purified antibody having a binding specificity for 11 alpha hydroxylase having an amino acid sequence as shown in SEQ ID NO: 2. Preferably the antibody binds to a protein region selected from the group consisting of (a) the N-terminal amino acids 1-10 of SEQ ID NO: 2; (b) the last 10 C-terminal amino acids of SEQ ID NO: 2; (c) amino acids SEQ ID NO: 23; (d) amino acids SEQ ID NO: 24; and (e) amino acids SEQ ID NO: 25.

10 Preferably the antibody is purified on a peptide column, wherein said peptide is selected from the group consisting of: (a) the N-terminal amino acids 1-10 of SEQ ID NO: 2; (b) the last 10 C-terminal amino acids of SEQ ID NO: 2; (c) amino acids SEQ ID NO: 23; (d) amino acids SEQ ID NO: 24; and (e) amino acids SEQ ID NO: 25.

15 The invention also provides for a purified polypeptide, the amino acid sequence of which is selected from the group consisting of SEQ ID NO: 26.

The invention also provides for a purified immunogenic polypeptide, the amino acid sequence of which comprises at least ten consecutive residues of SEQ ID NO: 6.

20 The invention also provides for an isolated and purified antibody having a binding specificity for 11 alpha hydroxylase having an amino acid sequence as shown in SEQ ID NO: 6. Preferably the antibody binds to a protein region selected from the group consisting of (a) the N-terminal amino acids 1-10 of SEQ ID NO: 6; (b) the last 10 C-terminal amino acids of SEQ ID NO: 6; and (c) amino acids SEQ ID NO: 26. More preferably, the antibody is purified on a peptide column, wherein said peptide is selected from the group consisting of: (a) the N-terminal amino acids 1-10 of SEQ ID NO: 6; (b) the last 10 C-terminal amino acids of SEQ ID NO: 6; and (c) amino acids SEQ ID NO: 26.

25

The invention also provides for a composition comprising an antibody described above in an effective carrier, vehicle, or auxiliary agent. It also provides for a composition comprising such an antibody and a solution. The antibody may be a polyclonal antibody. The antibody may also be a monoclonal antibody. The antibody may be conjugated to an immunoaffinity matrix. The invention also provides for a method of using an immunoaffinity matrix to purify a polypeptide from a biological fluid or cell lysate. Preferably the immunoaffinity matrix is

30

35

SEPHAROSE 4B. More preferably the method of using an immunoaffinity matrix to purify a polypeptide from a biological fluid or cell lysate uses SEPHAROSE 4B as an immunoaffinity matrix. More preferably, the method of using an immunoaffinity matrix to purify a polypeptide from a biological fluid or cell lysate. uses SEPHAROSE 4B as an immunoaffinity matrix.

5

The invention also provides for a method of using a peptide column to purify an antibody, wherein said peptide is selected from the group consisting of: (a) the N-terminal amino acids 1-10 of SEQ ID NO: 2; (b) the last 10 C-terminal amino acids of SEQ ID NO: 2; (c) amino acids SEQ ID NO: 23; (d) amino acids SEQ ID NO: 24; and (e) amino acids SEQ ID NO: 25.

10

The invention also provides for a method of using a peptide column to purify an antibody, wherein said peptide is selected from the group consisting of: (a) the N-terminal amino acids 1-10 of SEQ ID NO: 6; (b) the last 10 C-terminal amino acids of SEQ ID NO: 6; and (c) amino acids SEQ ID NO: 26.

15

The invention also provides for a method of detecting a first polypeptide in a biological fluid, wherein said first polypeptide is selected from the group consisting of 11 alpha hydroxylase and oxidoreductase, comprising the following steps: (a) contacting said fluid with a second polypeptide, having a binding specificity for said first polypeptide, and (b) assaying the presence of said second polypeptide to determine the level of said first polypeptide. Preferably, the second polypeptide is an antibody. More preferably, the second polypeptide is radiolabeled.

20

The invention also provides for a process for producing an isolated nucleic acid comprising hybridizing SEQ ID NO: 1 to genomic DNA in 6XSSC and 65°C and isolating the nucleic acid detected with SEQ ID NO: 1. The invention also provides for an isolated DNA nucleic acid prepared according to this process.

25

The invention also provides for an isolated nucleic acid that specifically hybridizes under highly stringent conditions to the complement of the sequence set forth in SEQ ID NO: 1.

30

The invention also provides for a process for producing an isolated nucleic acid comprising hybridizing SEQ ID NO: 5 to genomic DNA in 6XSSC and 65°C and isolating the nucleic acid detected with SEQ ID NO: 5. The invention also provides for an isolated DNA nucleic acid prepared according to this process.

The invention also provides for an isolated nucleic acid that specifically hybridizes under highly stringent conditions to the complement of the sequence set forth in SEQ ID NO: 5.

5 The invention also provides for a DNA construct which alters the expression of a 11 alpha hydroxylase gene not normally expressed in a cell when said DNA construct is inserted into chromosomal DNA of the cell, said DNA construct comprising: (a) a targeting sequence; (b) a regulatory sequence; and (c) the structural gene for a steroid 11 alpha hydroxylase. The invention also provides for a host cell harboring this DNA construct.

10 The invention also provides for a DNA construct which alters the expression of a 11 alpha hydroxylase gene not normally expressed in a cell when said DNA construct is inserted into chromosomal DNA of the cell, said DNA construct comprising: (a) a targeting sequence; (b) a regulatory sequence; and (c) the structural gene for a steroid oxidoreductase. The invention also provides for a
15 host cell harboring this DNA construct.

The invention also provides for use of a host cell harboring a cloned 11 alpha hydroxylase for the manufacture of a medicament for therapeutic application to treat heart disease, inflammation, arthritis, or cancer.

20 The invention also provides for a composition comprising from about 0.5-to about 500 g/L molasses, 0.5-50 g/L cornsteep liquid, 0.5-50 g/L KH_2PO_4 , 2.5-250 g/L NaCl, 2.5-250 g/L glucose, and 0.04-4 g/L progesterone, pH 3.5-7. Preferably, this composition is comprised of from about 10-250 g/L molasses, 1-25 g/L cornsteep liquid, 1-25 g/L KH_2PO_4 , 5-125 g/L NaCl, 5-125 g/L glucose, and 0.08-2 g/L progesterone, pH 4.5-6.5. More preferably, the composition is comprised of from
25 about 25-100 g/L molasses, 2.5-10 g/L cornsteep liquid, 2.5-10 g/L KH_2PO_4 , 12.5-50 g/L NaCl, 12.5-50 g/L glucose, and 0.2-0.8 g/L progesterone, pH 5.5-6.0. Most preferably the composition comprises about 50 g/L molasses, 5 g/L cornsteep liquid, 5 g/L KH_2PO_4 , 25 g/L NaCl, 25 g/L glucose, 20 g/L agar, and 0.4 g/L progesterone, pH 5.8.

30 The invention also provides for a semisolid formulation of any of the compositions described above, further comprising from about 4-100 g/L agar. Preferably the agar is at a concentration of from about 10-40 g/L agar. More preferably, the agar is about 20 g/L agar.

The invention also provides for the use of any of the compositions describe above to produce spores from the microorganism selected from the group consisting of *Aspergillus ochraceus*, *Aspergillus niger*, *Aspergillus nidulans*, *Rhizopus oryzae*, *Rhizopus stolonifer*, and *Trichothecium roseum*, *Fusarium oxysporum* *Rhizopus arrhizus*, *Monosporium olivaceum*, *Penicillium chrysogenum*, and *Absidia coerulea*.
 5 Preferably, the composition is used to produce spores from *Aspergillus ochraceus*.

Definitions

The following is a list of abbreviations and the corresponding meanings as used interchangeably herein:

- 10 11 alpha hydroxycanrenone = 11 alpha hydroxy-4-androstene-3,17-dione
 (C₂₂H₂₈O₄, MW 356.46)
- AcNPV = *Autographa californica* nuclear polyhedrosis virus, a member of
 the Baculoviridae family of insect viruses
- AD = androstenedione or 4-androstene-3,17-dione (C₂₂H₂₈O₃, MW 340.46)
- 15 aldadiene = canrenone
- Amp = ampicillin
- attTn7 = attachment site for Tn7 (a preferential site for Tn7 insertion into
 bacterial chromosomes)
- bacmid = recombinant baculovirus shuttle vector isolated from *E. coli*
- 20 Bluogal = halogenated indolyl-β-D-galactoside
- bp = base pair(s)
- Cam = chloramphenicol
- cDNA = complementary DNA
- DMF = N,N-dimethylformamide
- 25 ds = double-stranded
- eplerenone or epoxymexrenone = methyl hydrogen 9,11α-epoxy-17α-
 hydroxy-3-oxopregn-4-ene-7α,21-dicarboxylate, γ-lactone (MW 414.5)
- g = gram(s)
- Gen = gentamicin
- 30 hoxr = human oxidoreductase
- HPLC = high performance liquid chromatography
- hydroxycanrenone = 11 alpha- or 11 beta-hydroxycanrenone
- IPTG = isopropyl-β-D-thiogalactopyranoside
- Kan = kanamycin
- 35 kb = kilobase(s), 1000 bp(s)
- mb = megabase(s)

- Me = methyl
mg = milligram(s)
ml or mL = milliliter(s)
mm = millimeter
5 mM = millimolar
NMR = nuclear magnetic resonance
oxr = oxidoreductase
PCR = polymerase chain reaction
r = resistant or resistance
10 RP-HPLC = reverse phase high performance liquid chromatography
RT = room temperature
RT-PCR = reverse transcriptase polymerase chain reaction
s = sensitive
SDS-PAGE = sodium dodecyl sulfate polyacrylamide gel electrophoresis
15 Spc/Str = spectinomycin/streptomycin
Tet = tetracycline
Tn = transposon
ts = temperature-sensitive
U = units
20 ug or µg = microgram(s)
ul or µl = microliter(s)
X-gal = 5-bromo-3-chloro-indolyl-β-D-galactopyranoside
X-gluc = 5-bromo-3-chloro-indolyl-β-D-glucopyranoside

The following is a list definitions of various terms used herein:

- 25 The species "*Aspergillus ochraceus* NRRL 405" means the filamentous fungus *Aspergillus ochraceus* NRRL 405, accession number 18500, obtained from the American Type Culture Collection (ATCC). *A. ochraceus* NRRL 405 and *A. ochraceus* ATCC 18500 are the same strain, catalogued differently.

- 30 The term "amino acid(s)" means all naturally occurring L-amino acids, including norleucine, norvaline, homocysteine, and ornithine.

The term "degenerate" means that two nucleic acid molecules encode for the same amino acid sequences but comprise different nucleotide sequences.

The term "fragment" means a nucleic acid molecule whose sequence is shorter than the target or identified nucleic acid molecule and having the identical,

the substantial complement, or the substantial homologue of at least 10 contiguous nucleotides of the target or identified nucleic acid molecule.

The term "fusion protein" means a protein or fragment thereof that comprises one or more additional peptide regions not derived from that protein.

5 The term "probe" means an agent that is utilized to determine an attribute or feature (e.g. presence or absence, location, correlation, etc.) of a molecule, cell, tissue, or organism.

 The term "promoter" is used in an expansive sense to refer to the regulatory sequence(s) that control mRNA production. Such sequences include
10 RNA polymerase binding sites, enhancers, etc.

 The term "protein fragment" means a peptide or polypeptide molecule whose amino acid sequence comprises a subset of the amino acid sequence of that protein.

 The term "recombinant" means any agent (e.g., DNA, peptide, etc.), that is,
15 or results from, however indirectly, human manipulation of a nucleic acid molecule.

 The term "selectable or screenable marker genes" means genes whose expression can be detected by a probe as a means of identifying or selecting for transformed cells.

20 The term "specifically bind" means that the binding of an antibody or peptide is not competitively inhibited by the presence of non-related molecules.

 The term "specifically hybridizing" means that two nucleic acid molecules are capable of forming an anti-parallel, double-stranded nucleic acid structure.

 The term "substantial complement" means that a nucleic acid sequence
25 shares at least 80% sequence identity with the complement.

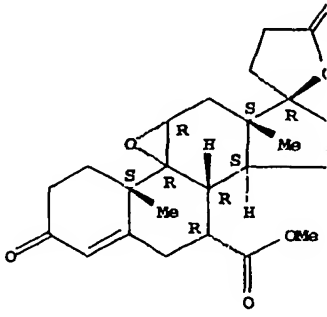
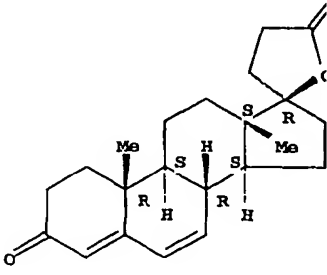
 The term "substantial fragment" means a nucleic acid fragment which comprises at least 100 nucleotides.

 The term "substantial homologue" means that a nucleic acid molecule shares at least 80% sequence identity with another.

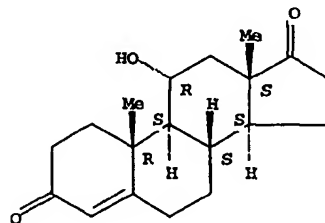
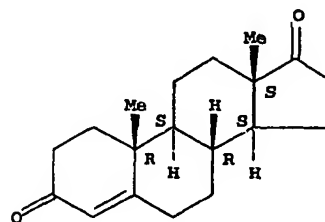
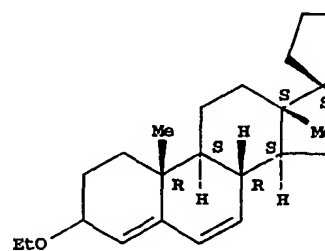
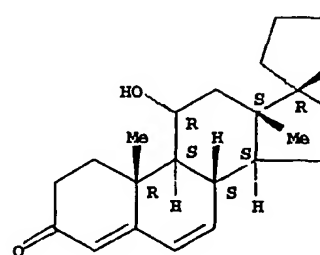
The term "substantially hybridizing" means that two nucleic acid molecules can form an anti-parallel, double-stranded nucleic acid structure under conditions (e.g., salt and temperature) that permit hybridization of sequences that exhibit 90% sequence identity or greater with each other and exhibit this identity for at least about a contiguous 50 nucleotides of the nucleic acid molecules.

The term "substantially-purified" means that one or more molecules that are or may be present in a naturally-occurring preparation containing the target molecule will have been removed or reduced in concentration.

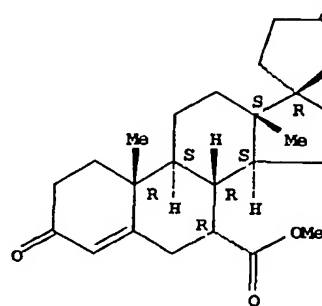
The following is a list of steroids, corresponding terms, and their structures, as used interchangeably herein:

#	Name	CA Index Name:	Other Names	Formula	Structure
1	Eplerenone	Pregn-4-ene-7,21-dicarboxylic acid, 9,11-epoxy-17-hydroxy-3-oxo-, γ -lactone, methyl ester, (7 α ,11 α ,17 α)-(9CI)	Spiro[9,11-epoxy-9H-cyclopenta[a]phenanthrene-17(2H),2'(3'H)-furan], pregn-4-ene-7,21-dicarboxylic acid deriv.; CGP 30083; Eplerenone; SC 66110	C24 H30 O6	
2	Aldadiene; Canrenone	Pregna-4,6-diene-21-carboxylic acid, 17-hydroxy-3-oxo-, γ -lactone, (17 α)-(9CI)	17 α -Pregna-4,6-diene-21-carboxylic acid, 17-hydroxy-3-oxo-, γ -lactone (6CI, 7CI, 8CI); Spiro[17H-cyclopenta[a]phenanthrene-17,2'(5'H)-furan], pregna-4,6-diene-21-carboxylic acid deriv.; 11614 R.P.; 17 β -Hydroxy-3-oxopregna-4,6-diene-21-carboxylic acid; 20-Spiroxa-4,6-diene-3,21-dione;	C22 H28 O3	

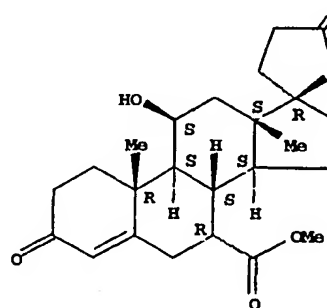
			Aldadiene; Canrenone; Phanurane; SC 9376; Spirolactone SC 14266	
3	11 α - Hydroxycanrenone	Pregna-4,6-diene-21-carboxylic acid, 11,17-dihydroxy-3-oxo-, γ -lactone, (11 α ,17 α)-(9CI)	11 α -Hydroxycanrenone	C22 H28 O4
5	Aldona ethyl enol ether	Pregna-4,6-diene-21-carboxylic acid, 3-ethoxy-17-hydroxy-, γ -lactone (9CI)	Spiro[17H-cyclopenta[a]phenanthrene-17,2'(5'H)-furan], pregna-4,6-diene-21-carboxylic acid deriv.; Aldona ethyl enol ether	C24 H34 O3
6	Androstenedione	Androst-4-ene-3,17-dione (8CI, 9CI)	Δ^4 -Androstene-3,17-dione; 17-Ketotestosterone; 3,17-Dioxoandrost-4-ene; Androstenedione; Fecundin; SKF 2170	C19 H26 O2
7	11 α -Hydroxyandrostenedione	Androst-4-ene-3,17-dione, 11-hydroxy-, (11 α)-(9CI)	Androst-4-ene-3,17-dione, 11 α -hydroxy- (8CI); 11 α -Hydroxyandrostenedione; 11 α -Hydroxyandrostenedione	C19 H26 O3



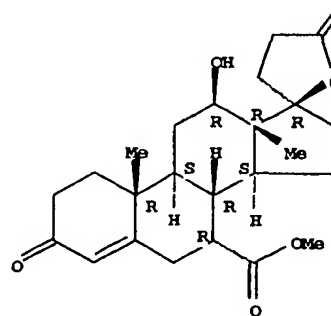
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|---|-----------|--|---|------------------|
| 8 | Mexrenone | Pregn-4-ene-7,21-dicarboxylic acid, 17-hydroxy-3-oxo-, γ -lactone, methyl ester, (7 α ,17 α)-(9CI) | Spiro[17H-cyclopenta[a]phenanthrene-17,2'(5'H)-furan], pregn-4-ene-7,21-dicarboxylic acid deriv.; Mexrenone; SC 25152; ZK 32055 | C24
H32
O5 |
|---|-----------|--|---|------------------|



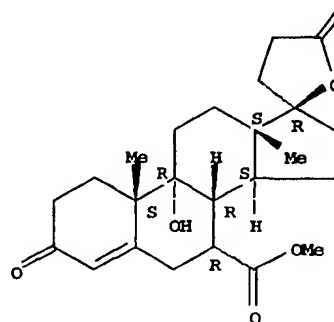
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|---|------------------------------|---|------------------------------|------------------|
| 9 | 11 β -Hydroxymexrenone | Pregn-4-ene-7,21-dicarboxylic acid, 11,17-dihydroxy-3-oxo-, γ -lactone, methyl ester, (7 α ,11 β ,17 α)-(9CI) | 11 β -Hydroxymexrenone | C24
H32
O6 |
|---|------------------------------|---|------------------------------|------------------|



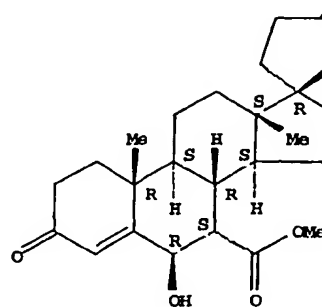
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|----|------------------------------|---|------------------------------|------------------|
| 10 | 12 β -Hydroxymexrenone | Pregn-4-ene-7,21-dicarboxylic acid, 12,17-dihydroxy-3-oxo-, γ -lactone, methyl ester, (7 α ,12 β ,17 α)-(9CI) | 12 β -Hydroxymexrenone | C24
H32
O6 |
|----|------------------------------|---|------------------------------|------------------|



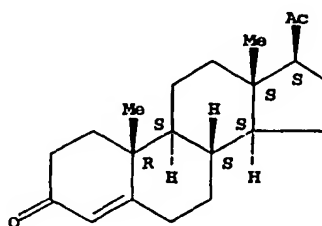
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|----|------------------------------|--|------------------------------|------------------|
| 11 | 9 α -Hydroxymexrenone | Pregn-4-ene-7,21-dicarboxylic acid, 9,17-dihydroxy-3-oxo-, 21,17-lactone, 7-methyl ester, (7 α ,17 α)-(9CI) | 9 α -Hydroxymexrenone | C24
H32
O6 |
|----|------------------------------|--|------------------------------|------------------|



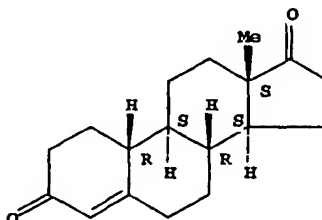
- 12 6 β -Hydroxymexrenone
Pregn-4-ene-7,21-dicarboxylic acid, 6,17-dihydroxy-3-oxo-, γ -lactone, methyl ester, (6 β ,7 α ,17 α)-(9CI)
Spiro[17H-cyclopenta[a]phenanthrene-17,2'(3'H)-furan], pregn-4-ene-7,21-dicarboxylic acid deriv.; 6 β -Hydroxymexrenone
C24
H32
O6



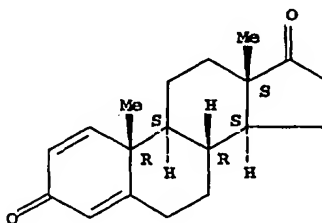
- 13 Progesterone
Pregn-4-ene-3,20-dione (9CI)
Progesterone (8CI); Δ 4-Pregnene-3,20-dione; and >70 other names
C21
H30
O2



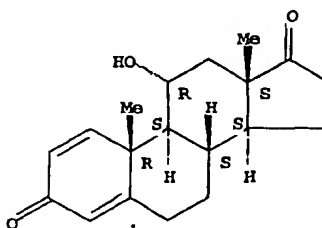
- 14 Estr-4-ene-3,17-dione
Estr-4-ene-3,17-dione (6CI, 8CI, 9CI)
(+)-19-Norandrost-4-ene-3,17-dione; Δ 4-Estrene-3,17-dione; 19-Norandrost-4-ene-3,17-dione
C18
H24
O2



- 15 Δ 1,4-androstadiene-3,17-dione (ADD)
Androsta-1,4-diene-3,17-dione (7CI, 8CI, 9CI)
 Δ 1,4-Androstadiene-3,17-dione; 1-Dehydroandrostenedione; Androstadienedione; Androstane-1,4-diene-3,17-dione
C19
H24
O2



- 16 11 α -Hydroxyandrost-1,4-diene-3,17-dione (11 α -hydroxy-, (11 α)-(9CI)
Androsta-1,4-diene-3,17-dione, 11 α -hydroxy- (6CI, 7CI, 8CI); 11 α -Hydroxyandrost-1,4-diene-3,17-dione; Kurchinin
C19
H24
O3



- 17 aldona

Compound 5 (aldona ethyl enol ether with O= in place of EtO- at position

18	mexrenone 6,7-bislactone	Compound 12 with cyclic bis-lactone ring (-O-C=O-) formed between carbons at positions 6 and 7 (See U 5,981,744 for discussion of similar lactone rings)
19	11 alpha hydroxy mexrenone 6,7-bislactone	11 alpha hydroxy version of Compound 18
20	mexrenone 7,9-bislactone	Compound 11 with cyclic bis-lactone ring (-O-C=O-) formed between carbons at positions 7 and 9 (See U 5,981,744 for discussion of similar lactone rings)
21	11 alpha hydroxy mexrenone 7,9-bislactone	11 alpha hydroxy version of Compound 20

Figure 1 - Nucleotide and protein sequence of *Aspergillus ochraceus* 11 alpha hydroxylase

The nucleotide and protein sequences of *Aspergillus ochraceus* 11 alpha hydroxylase (SEQ ID NO: 1, SEQ ID NO: 2, respectively) are displayed.

Figure 2 - Nucleotide and protein sequence of human oxidoreductase

The nucleotide and protein sequences of human oxidoreductase (SEQ ID NO: 3, SEQ ID NO: 4, respectively) are displayed. The predicted amino acid sequence of human oxidoreductase independently cloned from a cDNA library prepared by RT-PCR using the RNA from a human HepG2 cells as a template, as disclosed in this specification, matches that previously reported by three different laboratories. The GenBank accession numbers for these loci include A60557 (NADPH-ferrihemoprotein reductase (EC 1.6.2.4) - human); AAG09798 (NADPH-cytochrome P450 reductase [Homo sapiens]), and P16435 (NADPH-CYTOCHROME P450 REDUCTASE (CPR) (P450R)).

The amino acid sequence of AAB21814 (cytochrome P450 reductase [EC 1.6.2.4] [human, placenta, Peptide Partial, 676 aa]), differs from human oxidoreductase A60557 and P16435 at 4 residues: A→V at 500, F→L at 518, V→W at 537, and A→H at 538. The initial methionine is also missing from AAB21814. The cognate nucleic acid for AAB21814 (S90469 [cytochrome P450 reductase

[human, placenta, mRNA Partial, 2403 nt]) lacks the ATG codon for the initial methionine and includes a C→T change at 1496, a C→A, change at 1551, and a frameshift due to a missing G at 1605 which is resolved by the addition of a T at 1616.

- 5 References for these loci are as follows: A60557 [Yamano,S., Aoyama,T., McBride,O.W., Hardwick,J.P., Gelboin,H.V. and Gonzalez,F.J. Human NADPH-P450 oxidoreductase: complementary DNA cloning, sequence and vaccinia virus-mediated expression and localization of the CYPOR gene to chromosome 7 Mol. Pharmacol. 36 (1), 83-88 (1989)]; AAG09798 [Czerwinski,M., Sahni,M., Madan,A. and Parkinson,A. Polymorphism of human CYPOR: Expression of new allele. Unpublished, Direct Submission], and P16435 [Haniu,M., McManus,M.E., Birkett,D.J., Lee,T.D. and Shively,J.E. Structural and functional analysis of NADPH-cytochrome P-450 reductase from human liver: complete sequence of human enzyme and NADPH-binding sites. Biochemistry 28 (21), 8639-8645 (1989)]; AAB21814 [Shephard,E.A., Palmer,C.N., Segall,H.J. and Phillips,I.R. Quantification of cytochrome P450 reductase gene expression in human tissues. Arch. Biochem. Biophys. 294 (1), 168-172 (1992)]; S90469 [Shephard,E.A., Palmer,C.N., Segall,H.J. and Phillips,I.R. Quantification of cytochrome P450 reductase gene expression in human tissues. Arch. Biochem. Biophys. 294 (1), 168-172 (1992)].

Figure 3 - Nucleotide and protein sequence of *Aspergillus ochraceus* oxidoreductase

The nucleotide and protein sequences of *Aspergillus ochraceus* 11 oxidoreductase (SEQ ID NO: 5, SEQ ID NO: 6, respectively) are displayed.

Figure 4 - Amino acid homology alignment of *A. ochraceus* 11 alpha hydroxylase with the top 10 BLAST hits from GenBank

- Aspergillus ochraceus* steroid 11 alpha hydroxylase (SEQ ID NO: 02), cloned into plasmid pMON45624 (SEQ ID NO: 01), was aligned with related enzymes found in GenBank using the BLASTP program that implements a heuristic matching algorithm (Altschul et al., *J Mol Biol* Oct 5;215(3):403-10, 1990). The GenBank accession numbers (its probable function, [genus and species]) for the top 10 matches are as follows: CAA75565 (cytochrome P450 monooxygenase [*Gibberella fujikuroi*]; CAB91316 (probable cytochrome P450 monooxygenase (lovA) [*Neurospora crassa*]); CAB56503 (cytochrome P450 [*Catharanthus roseus*]); AAB94588 (CYP71D10p [*Glycine max*]); CAA75566 (cytochrome P450

monooxygenase [*Gibberella fujikuroi*]; AAD34552 (cytochrome P450 monooxygenase [*Aspergillus terreus*]); CAA75567 (cytochrome P450 monooxygenase [*Gibberella fujikuroi*]); CAA76703 (cytochrome P450 [*Gibberella fujikuroi*]); CAA57874 (unnamed protein product [*Fusarium oxysporum*]);
 5 CAA91268 (similar to cytochrome P450-cDNA EST yk423b11.3 comes from this gene [*Caenorhabditis elegans*]).

References for these loci are as follows: CAA75565 [Tudzynski,B. and Holter,K., Gibberellin biosynthetic pathway in *Gibberella fujikuroi*: evidence for a gene cluster. *Fungal Genet. Biol.* **25** (3), 157-170 (1998)]; CAB91316 [Schulte,U., Aign,V., Hoheisel,J., Brandt,P., Fartmann,B., Holland,R., Nyakatura,G., Mewes,H.W. and Mannhaupt,G., Unpublished]; CAB56503 [Schroeder,G., Unterbusch,E., Kaltenbach,M., Schmidt,J., Strack,D. and Schroeder,J. Light-induced cytochrome P450-dependent enzyme in indole alkaloid biosynthesis: tabersonine 16-hydroxylase *FEBS Lett.* **458**, 97-102 (1999)]; AAB94588
 10 [Siminszky,B., Corbin,F.T., Ward,E.R., Fleischmann,T.J. and Dewey,R.E. Expression of a soybean cytochrome P450 monooxygenase cDNA in yeast and tobacco enhances the metabolism of phenylurea herbicides. *Proc. Natl. Acad. Sci. U.S.A.* **96** (4), 1750-1755 (1999)]; CAA75566 [Tudzynski,B. and Holter,K. Gibberellin biosynthetic pathway in *Gibberella fujikuroi*: evidence for a gene cluster. *Fungal Genet. Biol.* **25** (3), 157-170 (1998)]; AAD34552 [Kennedy,J., Auclair,K., Kendrew,S.G., Park,C., Vederas,J.C. and Hutchinson,C.R. Accessory Proteins Modulate Polyketide Synthase Activity During Lovastatin Biosynthesis. *Science* (1999) In press]; CAA75567 [Tudzynski,B. and Holter,K. Gibberellin biosynthetic pathway in *Gibberella fujikuroi*: evidence for a gene cluster. *Fungal Genet. Biol.* **25** (3), 157-170 (1998)]; CAA76703 [Tudzynski,B. and Hoelter,K. Characterization of P450 monooxygenase genes from *Gibberella fujikuroi*. Unpublished]; CAA57874 [Mouyna,I. and Brygoo,Y. Disruption of a *Fusarium oxysporum f.sp. elaeidis* cytochrome P450 gene by a repetitive sequence. Unpublished]; and CAA91268 [No Authors. Genome sequence of the nematode *C. elegans*: a platform for investigating biology. The *C. elegans* Sequencing Consortium. *Science* **282** (5396), 2012-2018 (1998) [Published errata appear in *Science* 1999 Jan 1;**283**(5398):35 and 1999 Mar 26;**283**(5410):2103 and 1999 Sep 3;**285**(5433):1493]].
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Figure 5 - Phylogenetic tree showing the relatedness of *Aspergillus ochraceus* 11 alpha hydroxylase to the top 10 BLAST hits from GenBank

A phylogenetic tree displaying the genetic relatedness of *Aspergillus ochraceus* steroid 11 alpha hydroxylase, cloned into plasmid pMON45624, was aligned with related enzymes found in GenBank. BLAST was used to find the related enzymes within GenBank, and ClustalW was used generate the multiple sequence alignment and phylogenetic tree depicted in this figure. Descriptions of the GenBank accession numbers used as labels in the figure are the same as that described above for the legend to Figure 4.

Figure 6 - Percent homology between *Aspergillus ochraceus* 11 alpha hydroxylase and the top 10 BLAST hits from GenBank

The percent homology between *Aspergillus ochraceus* steroid 11 alpha hydroxylase and the top 10 enzymes found in GenBank using BLAST was calculated using CLUSTAL (Thompson et al., *Comput. Appl. Biosci.* 10:19-29, 1994).

Figure 7 - Amino acid homology alignment of *Aspergillus ochraceus* and human oxidoreductase to NADPH cytochrome P450 reductases from *A. niger*, mouse, and *S. cerevisiae*

The amino acid sequences of *Aspergillus ochraceus* steroid oxidoreductase (SEQ ID NO: 06) cloned into plasmid pMON45632 (SEQ ID NO: 05), and human oxidoreductase (SEQ ID NO: 03), cloned into plasmid pMON45605 (SEQ ID NO: 04) were aligned with related enzymes from *A. niger*, mouse, and *S. cerevisiae*, as described above. The GenBank accession numbers (probable function, [genus and species]) are as follows: BAA02936 (NADPH-cytochrome P450 reductase precursor [*Saccharomyces cerevisiae*]); CAA81550 NADPH cytochrome P450 oxidoreductase [*Aspergillus niger*]; P16435 (NADPH-CYTOCHROME P450 REDUCTASE (CPR) (P450R) [human]); BAA04496 (NADPH-cytochrome P450 oxidoreductase [*Mus musculus*]).

References for these loci are as follows: BAA02936 [Yabusaki,Y., Murakami,H. and Ohkawa,H. Primary structure of *Saccharomyces cerevisiae* NADPH-cytochrome P450 reductase deduced from nucleotide sequence of its cloned gene. *J. Biochem.* 103 (6), 1004-1010 (1988)]; CAA81550 [van den Brink,J., van Zeijl,C., van den Hondel,C. and van Gorcom,R. Cloning and characterization of the NADPH cytochrome P450 oxidoreductase (cprA) gene of *Aspergillus niger*.

Unpublished]; P16435 [Haniu,M., McManus,M.E., Birkett,D.J., Lee,T.D. and Shively,J.E. Structural and functional analysis of NADPH-cytochrome P-450 reductase from human liver: complete sequence of human enzyme and NADPH-binding sites *Biochemistry* 28 (21), 8639-8645 (1989)]; BAA04496 [Ohgiya,S., Shinriki,N., Kamataki,T. and Ishizaki,K. Mouse NADPH-cytochrome P-450 oxidoreductase: molecular cloning and functional expression in yeast. *Biophys. Acta* 1186 (1-2), 137-141 (1994)].

Figure 8 - Amino acid homology alignment of *A. ochraceus* oxidoreductase to NADPH cytochrome P450 reductases from *A. niger*, mouse, and *S. cerevisiae*

The amino acid sequence of *Aspergillus ochraceus* steroid oxidoreductase (SEQ ID NO: 06) cloned into plasmid pMON45632 (SEQ ID NO: 05), was aligned with related fungal enzymes from *A. niger* and *S. cerevisiae*, as described above. Descriptions of the GenBank accession numbers used as labels in the figure are the same as that described above for the legend to Figure 7, above.

Figure 9 - Phylogenetic tree showing the relatedness of *Aspergillus ochraceus* and human oxidoreductase to reductases from *A. niger*, yeast, and mouse.

A phylogenetic tree displaying the genetic relatedness of *Aspergillus ochraceus* oxidoreductase (SEQ ID NO: 06), cloned into plasmid pMON45632 (SEQ ID NO: 05), was aligned with related enzymes. BLAST was used to find the related enzymes within GenBank, and ClustalW was used generate the multiple sequence alignment and phylogenetic tree depicted in this figure. Descriptions of the GenBank accession numbers used as labels in the figure are the same as that described above for the legend to Figure 7, above.

Figure 10 - Percent identity between *Aspergillus ochraceus* oxidoreductase and reductases from *A. niger*, yeast, and mouse.

The percent identity between *Aspergillus ochraceus* oxidoreductase and the oxidoreductases from *A. niger*, yeast, and mouse was calculated using Clustal W and Boxshade.

Figure 11 - Alignment of human oxidoreductase with top 4 hits from SwissProt

The amino acid sequences of human steroid oxidoreductase (SEQ ID NO: 04), cloned into plasmid pMON45605 (SEQ ID NO: 03), which corresponds to the amino acid sequence of the corrected sequence reported for P16435 below, was aligned with the top 4 hits from the SWISSPROT protein sequence database, as described above. The SWISSPROT accession numbers [locus] [common name] and species)) probable function) are as follows: P16435 {NCPR_HUMAN} [human] NADPH-CYTOCHROME P450 REDUCTASE; P00389 {NCPR_RABIT} [rabbit] NADPH-CYTOCHROME P450 REDUCTASE; P00388 {NCPR_RAT} [rat] NADPH-CYTOCHROME P450 REDUCTASE; P37040 {NCPR_MOUSE} [mouse] NADPH-CYTOCHROME P450 REDUCTASE; P04175 {NCPR_PIG} [pig] (NADPH-CYTOCHROME P450 REDUCTASE.

References for these loci are as follows: P16435 [Haniu,M., McManus,M.E., Birkett,D.J., Lee,T.D. and Shively,J.E. Structural and functional analysis of NADPH-cytochrome P-450 reductase from human liver: complete sequence of human enzyme and NADPH-binding sites. *Biochemistry* 28 (21), 8639-8645 (1989)]; P00389 [Katagiri,M., Murakami,H., Yabusaki,Y., Sugiyama,T., Okamoto,M., Yamano,T. and Ohkawa,H. Molecular cloning and sequence analysis of full-length cDNA for rabbit liver NADPH-cytochrome P-450 reductase mRNA. *J. Biochem.* 100 (4), 945-954 (1986)]; P00388 [Porter,T.D. and Kasper,C.B. Coding nucleotide sequence of rat NADPH-cytochrome P-450 oxidoreductase cDNA and identification of flavin-binding domains. *Proc. Natl. Acad. Sci. U.S.A.* 82 (4), 973-977 (1985)]; P37040 [Ohgiya,S., Shinriki,N., Kamataki,T. and Ishizaki,K. Mouse NADPH-cytochrome P-450 oxidoreductase: molecular cloning and functional expression in yeast. *Biochim. Biophys. Acta* 1186 (1-2), 137-141 (1994)]; P04175 [Haniu,M., Iyanagi,T., Miller,P., Lee,T.D. and Shively,J.E. Complete amino acid sequence of NADPH-cytochrome P-450 reductase from porcine hepatic microsomes. *Biochemistry* 25 (24), 7906-7911 (1986)].

Figure 12 - Phylogenetic tree showing the relatedness of human oxidoreductases with top 4 hits from SwissProt

A phylogenetic tree displaying the genetic relatedness of human oxidoreductase (SEQ ID NO: 04), cloned into plasmid pMON45604 (SEQ ID NO: 03), was aligned with related enzymes found in SWISSPROT. BLAST was used to find the related enzymes within SWISSPROT, and ClustalW was used generate

the multiple sequence alignment and phylogenetic tree depicted in this figure. Descriptions of the SWISSPROT accession numbers used as labels in the figure are the same as that described above for the legend to Figure 11, above.

Figure 13 – Percent identity between human oxidoreductase and top 4 hits from SwissProt

The percent identity between human oxidoreductase and the top 4 hits found in SWISSPROT was calculated using Clustal W and Boxshade.

Figure 14: Expression of *Aspergillus ochraceus* 11 alpha hydroxylase in transfected Sf9 insect cells

Baculovirus-infected insect cells expressing *Aspergillus ochraceus* 11 alpha hydroxylase were harvested at 25 and 48 hours post infection and microsomal membrane fractions were prepared and separated by SDS-polyacrylamide gel electrophoresis. The proteins in the gel were electrophoretically transferred to 0.2 um nitrocellulose membrane (Schleicher & Schuell Grimsehlstrasse 23 37574 Einbeck Germany) and probed with antibodies GN-1187 and GN-1188 prepared from peptide 11aOH peptide 2 CRQILTPYIHKRSLKGTDD (SEQ ID NO: 24).

Figure 15: Expression of *Aspergillus ochraceus* P450 oxidoreductase in transfected Sf9 insect cells

Baculovirus-infected insect cells expressing *Aspergillus ochraceus* 11 oxidoreductase were harvested at 25 and 48 hours post infection and microsomal membrane fractions were prepared and separated by SDS-polyacrylamide gel electrophoresis. The proteins in the gel were electrophoretically transferred to 0.2 um nitrocellulose membrane (Schleicher & Schuell Grimsehlstrasse 23 37574 Einbeck Germany) and probed with antibodies GN-2023 and GN-12024 prepared from oxr peptide 1 CTYWAVAKDPYASAGPAMNG (SEQ ID NO: 26).

Figure 16 – Conversion of androstenedione to 11 alpha hydroxy androstenedione monitored by HPLC

Microsomal and mitochondrial subcellular fractions were prepared from insect cells co-infected with recombinant baculoviruses expressing recombinant *Aspergillus ochraceus* 11 alpha hydroxylase and human oxidoreductase cloned from HepG2 cell RNA. The subcellular fractions were incubated with 250 μM androstenedione (AD) in the presence of an NADPH-generating system for 120

minutes, and the resulting products were separated by HPLC and monitored by ultraviolet detection at 247 nm. Hydroxylase activity was found in the microsomal fraction, as expected, but also appeared in the mitochondrial fraction. These results suggest that the 11 alpha hydroxylase may have a tendency to stick to membranes in disrupted cells, or that the separation of the subcellular fractions in this experiment was insufficient. Panel A illustrates a reaction carried out using enzyme prepared from a mitochondrial fraction. The peak in panel A that elutes after AD appears to be testosterone. When a microsomal fraction was used, almost as much AD was converted to 11 alpha hydroxy AD, but relatively more testosterone was also produced. Panel B illustrates the same reaction carried out for 120 minutes without a source of enzyme. Panel C illustrates an HPLC tracing with 11 α -hydroxyandrostenedione standard added to incubation buffer.

Detailed Description of the Invention

The present invention encompasses enzymes that facilitate the biosynthesis of steroid molecules, particularly enzymes possessing cytochrome P450 or oxidoreductase activities. The present invention is directed, in part, to the isolation of a nucleic acid encoding *Aspergillus ochraceus* 11 alpha hydroxylase, which exhibits sequence homology to the highly conserved residues that correspond to cytochrome P450 enzymes. It also directed to the isolation of nucleic acids encoding human and *Aspergillus ochraceus* oxidoreductase. Biological activities of the cloned hydroxylases and oxidoreductases of the present invention can be determined by a variety of assays, including incubation of steroid substrates in the presence of microsomes prepared from recombinant baculovirus-infected insect cells and monitoring the conversion to their 11 alpha hydroxy-counterparts by high pressure liquid chromatography (HPLC). The present invention, comprising novel 11 alpha hydroxylase and oxidoreductase nucleic acids, proteins, peptides, homologues, and fragments of either, provides new and advantageous methods to convert steroid intermediates to their 11 alpha hydroxy counterparts.

The present invention also includes the DNA sequences which code for the 11 alpha hydroxylases and oxidoreductases, DNA sequences which are substantially similar and perform substantially the same function, and DNA sequences which differ from the DNAs encoding the hydroxylases and oxidoreductases of the invention only due to the degeneracy of the genetic code. Also included in the present invention are the oligonucleotide intermediates used to construct mutated versions of these DNAs and the polypeptides encoded by these oligonucleotides and mutant DNAs.

5 The present invention also includes antibodies which bind specifically to *A. ochraceus* 11 alpha hydroxylase or *A. ochraceus* oxidoreductase, including anti-peptide antibodies, methods of using these anti-peptide antibodies to purify these and other related polypeptides, methods of using the purified polypeptides to generate polyclonal or monoclonal antibodies to the full-length polypeptides, and methods of using antibodies to the full-length polypeptides to assess the presence of the polypeptides in recombinant and non-recombinant host cells. The antibodies can be used to identify related polypeptides in any of a variety of host organisms that possess the biological activities associated with these polypeptides.

10 Among the preferred organisms that can be used in this hydroxylation step are *Aspergillus ochraceus* NRRL 405, *Aspergillus ochraceus* ATCC 18500, *Aspergillus niger* ATCC 16888 and ATCC 26693, *Aspergillus nidulans* ATCC 11267, *Rhizopus oryzae* ATCC 11145, *Rhizopus stolonifer* ATCC 6227b, *Streptomyces fradiae* ATCC 10745, *Bacillus megaterium* ATCC 14945, 15 *Pseudomonas cruciviae* ATCC 13262, and *Trichothecium roseum* ATCC 12543. Other preferred organisms include *Fusarium oxysporum* f. sp. *cepa* ATCC 11171 and *Rhizopus arrhizus* ATCC 11145.

Other organisms that have exhibited activity for this reaction include *Absidia coerula* ATCC 6647, *Absidia glauca* ATCC 22752, *Actinomucor elegans* 20 ATCC 6476, *Aspergillus flavipes* ATCC 1030, *Aspergillus fumigatus* ATCC 26934, *Beauveria bassiana* ATCC 7159 and ATCC 13144, *Botryosphaeria obtusa* IMI 038560, *Calonectria decora* ATCC 14767, *Chaetomium cochliodes* ATCC 10195, *Corynespora cassiicola* ATCC 16718, *Cunninghamella blakesleeana* ATCC 8688a, *Cunninghamella echinulata* ATCC 3655, *Cunninghamella elegans* ATCC 9245, 25 *Curvularia clavata* ATCC 22921, *Curvularia lunata* ACTT 12071, *Cylindrocarpon radiculicola* ATCC 1011, *Epicoccum humicola* ATCC 12722, *Gongronella butleri* ATCC 22822, *Hypomyces chrysospermus*, *Mortierella isabellina* ATCC 42613, *Mucor mucedo* ATCC 4605, *Mucor griseocyanus* ATCC 1207A, *Myrothecium verrucaria* ATCC 9095, *Nocardia corallina*, *Paecilomyces carneus* ATCC 46579, 30 *Penicillium patulum* ATCC 24550, *Pithomyces atrolivaceus* IFO 6651, *Pithomyces cynodontis* ATCC 26150, *Pycnosporium* sp. ATCC 12231, *Saccharopolyspora erythrae* ATCC 11635, *Sepedonium chrysospermum* ATCC 13378, *Stachylidium bicolor* ATCC 12672, *Streptomyces hygroscopicus* ATCC 27438, *Streptomyces purpurascens* ATCC 25489, *Syncephalastrum racemosum* ATCC 18192, 35 *Thamnostylum piriforme* ATCC 8992, *Thielavia terricola* ATCC 13807, and *Verticillium theobromae* ATCC 12474.

Additional organisms that may be expected to show activity for the 11 α hydroxylation include *Cephalosporium aphidicola* (*Phytochemistry* (1996), 42(2), 411-415), *Cochliobolus lunatas* (*J. Biotechnol.* (1995), 42(2), 145-150), *Tieghemella orchidis* (*Khim.-Farm.Zh.* (1986), 20(7), 871- 876), *Tieghemella hyalospora* (*Khim.-Farm.Zh.* (1986), 20(7), 871-876), *Monosporium olivaceum* (*Acta Microbiol. Pol.*, Ser. B. (1973), 5(2), 103-110), *Aspergillus ustus* (*Acta Microbiol. Pol.*, Ser. B. (1973), 5(2), 103-110), *Fusarium graminearum* (*Acta Microbiol. Pol.*, Ser. B. (1973), 5(2), 103-110), *Verticillium glaucum* (*Acta Microbiol. Pol.*, Ser. B. (1973), 5(2), 103-110), and *Rhizopus nigricans* (*J. Steroid Biochem.* (1987), 28(2), 197-201).

Figure 1 sets forth the nucleotide and protein sequence of *Aspergillus ochraceus* 11 alpha hydroxylase (SEQ ID NO: 1, SEQ ID NO: 2, respectively). Figure 2 sets forth the nucleotide and protein sequence of human oxidoreductase (SEQ ID NO: 3, SEQ ID NO: 4, respectively). Figure 3 sets forth the nucleotide and protein sequence of *Aspergillus ochraceus* oxidoreductase (SEQ ID NO: 5, SEQ ID NO: 6, respectively).

Figure 4 sets forth an amino acid homology alignment of *A. ochraceus* 11 alpha hydroxylase cloned in pMON45624 and aligned with related enzymes found in GenBank using BLAST. Figure 5 is a phylogenetic tree showing the this relationship graphically. Figure 6 shows the percent homology between *Aspergillus ochraceus* steroid 11 alpha hydroxylase and the top 10 enzymes found in GenBank using BLAST, calculated using Clustal W and Boxshade.

Figure 7 sets forth the amino acid homology of *Aspergillus ochraceus* and human oxidoreductase to NADPH cytochrome P450 reductases from *A. niger*, mouse, and *S. cerevisiae* (yeast). Figure 8 sets forth the amino acid alignment for *A. ochraceus*, *A. niger*, and *S. cerevisiae* oxidoreductases. Figure 9 is a phylogenetic tree showing the relatedness of *Aspergillus ochraceus* and human oxidoreductase to reductases from *A. niger*, yeast, and mouse. Figure 10 shows the percent homology between *Aspergillus ochraceus* steroid 11 alpha hydroxylase and the oxidoreductases from *A. niger*, yeast, and mouse, calculated using Clustal W and Boxshade.

Figure 11 - Alignment of human oxidoreductase with top 4 hits from SwissProt. Figure 12 sets forth a phylogenetic tree displaying the genetic relatedness of human oxidoreductase, to these hits. Figure 13 shows the percent identity between human oxidoreductase and top 4 hits from SwissProt.

Figure 14 sets forth an immunoblot illustrating expression of *Aspergillus ochraceus* P450 11 alpha hydroxylase in baculovirus-infected insect cells harvested at 25 and 48 hours post infection. The nitrocellulose membrane was probed with a 1:1 mixture of antibodies prepared from two rabbits immunized with a conjugated synthetic peptide, 11aOH peptide 2 (SEQ ID NO 24).

Figure 15 sets forth an immunoblot illustrating expression of *Aspergillus ochraceus* P450 oxidoreductase in baculovirus-infected insect cells harvested at 25 and 48 hours post infection. The nitrocellulose membrane was probed with a 1:1 mixture of antibodies prepared two rabbits immunized with a conjugated synthetic peptide, oxr peptide 1 (SEQ ID NO 26).

Figure 16 sets forth an HPLC tracing illustrating the conversion of androstenedione (AD) to its 11 alpha hydroxy counterpart after incubating AD with subcellular fractions prepared from baculovirus-infected insect cells expressing *Aspergillus ochraceus* 11 alpha hydroxylase and human oxidoreductase.

Cloning techniques

Genetic engineering techniques now standard in the art (U.S. Patent 4,935,233 and Sambrook et al., "Molecular Cloning A Laboratory Manual", Cold Spring Harbor Laboratory, 1989) may be used in the construction of the DNA sequences of the present invention. One such method is cassette mutagenesis (Wells et al., *Gene* 34:315-323, 1985) in which a portion of the coding sequence in a plasmid is replaced with synthetic oligonucleotides that encode the desired amino acid substitutions in a portion of the gene between two restriction sites.

Pairs of complementary synthetic oligonucleotides encoding the desired gene can be made and annealed to each other. The DNA sequence of the oligonucleotide would encode sequence for amino acids of desired gene with the exception of those substituted and/or deleted from the sequence.

Plasmid DNA can be treated with the chosen restriction endonucleases then ligated to the annealed oligonucleotides. The ligated mixtures can be used to transform competent *E. coli* cells which will confer resistance to an appropriate antibiotic. Single colonies can be picked and the plasmid DNA examined by restriction analysis or by DNA sequencing to identify plasmids with the desired genes.

Cloning of DNA sequences encoding novel proteins and fusion proteins may be accomplished by the use of intermediate vectors. Linkers and adapters can be used to join DNA sequences, and to replace lost sequences, where a restriction site is internal to the region of interest. DNA encoding a single polypeptide or a fusion protein (comprising a first polypeptide, a peptide linker, and a second polypeptide) is inserted into a suitable expression vector which is then transformed or transfected into appropriate bacterial, fungal, insect, or mammalian host cells. The transformed organism or host cell line is grown and the recombinant protein isolated by standard techniques. Recombinant fusion proteins have all or a portion of a first protein joined by a linker region to a all or a portion of second protein.

Hybridization

Nucleic acid molecules and fragment nucleic acid molecules encoding 11 alpha hydroxylases or oxidoreductases can specifically hybridize with other nucleic acid molecules. Two nucleic acid molecules are said to be capable of specifically hybridizing to one another if the two molecules are capable of forming an anti-parallel, double-stranded nucleic acid structure. A nucleic acid molecule is said to be the "complement" of another nucleic acid molecule, if they exhibit complete complementarity. Molecules exhibit "complete complementarity" when every nucleotide of one of the molecules is complementary to a nucleotide of the other. Two molecules are "minimally complementary" if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under at least conventional "low-stringency" conditions. Similarly, the molecules are "complementary" if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under conventional "high-stringency" conditions. Conventional stringency conditions are described by Sambrook, *et al.*, *Molecular Cloning*, A Laboratory Manual, 2nd Ed., Cold Spring Harbor Press, Cold Spring Harbor, New York (1989), and by Haymes, *et al.* *Nucleic Acid Hybridization, A Practical Approach*, IRL Press, Washington, DC, 1985). Departures from complete complementarity are therefore permissible, as long as such departures do not completely preclude the capacity of the molecules to form a double-stranded structure.

Appropriate stringency conditions which promote DNA hybridization are well known to those skilled in the art, or can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y., 6.3.1-6.3.6, (1989). Basic conditions would include, for example, 6X sodium saline citrate (SSC) at about 45°C, followed by a wash of 2X SSC at 50°C. Stringency can be varied, for example, by altering

the salt concentration in the wash step from about 2X SSC at 50°C (moderately low stringency) to about 0.2X SSC at 50°C (high stringency). Stringency can also be altered by changing the temperature in the wash step, from room temperature, about 22°C (low stringency conditions), to about 65°C (high stringency conditions).

5 Both temperature and salt may be varied, or either the temperature or the salt concentration may be held constant while the other variable is changed.

Expression vectors

Another aspect of the present invention includes plasmid DNA vectors for use in the expression of these novel hydroxylases and oxidoreductases. These

10 vectors contain the novel DNA sequences described above which code for the novel polypeptides of the invention. Appropriate vectors which can transform microorganisms or cell lines capable of expressing the hydroxylases and oxidoreductases include expression vectors comprising nucleotide sequences coding for the hydroxylases and oxidoreductases joined to transcriptional and

15 translational regulatory sequences which are selected according to the host cells used.

Vectors incorporating modified sequences as described above are included in the present invention and are useful in the production of the hydroxylases and oxidoreductases. The vector employed in the method also contains selected

20 regulatory sequences in operative association with the DNA coding sequences of the invention and which are capable of directing the replication and expression thereof in selected host cells.

Methods for producing the hydroxylases and oxidoreductases is another aspect of the present invention. The method of the present invention involves

25 culturing suitable cells or cell lines, which has been transformed with a vector containing a DNA sequence encoding novel hydroxylases and oxidoreductases. Suitable cells or cell lines may be bacterial cells. For example, various strains of *E. coli* are well-known as host cells in the field of biotechnology. Examples of such strains include *E. coli* strains DH5 alpha, DH10B and MON105 (Obukowicz et al.,

30 *Applied Environmental Microbiology* 58: 1511-1523, 1992). Also included in the present invention is the expression of the hydroxylases and oxidoreductases utilizing a chromosomal expression vector for *E. coli* based on the bacteriophage Mu (Weinberg et al., *Gene* 126: 25-33, 1993). Various other strains of bacteria, including the Enteric bacteria (e.g., *Salmonella* sp.) and *B. subtilis*, may also be

35 employed in this method.

When expressed in the *E. coli* cytoplasm, the gene encoding the proteins of the present invention may also be constructed such that at the 5' end of the gene codons are added to encode Met²-Ala¹, Met²-Ser¹, Met²-Cys¹, or Met¹ at the N-terminus of the protein. The N termini of proteins made in the cytoplasm of *E. coli* are affected by post-translational processing by methionine aminopeptidase (Ben Bassat et al., *J. Bacteriol.* 169:751-757, 1987), and possibly by other peptidases, so that upon expression the methionine is cleaved off the N-terminus. The proteins of the present invention may include polypeptides having Met¹, Ala¹, Ser¹, Cys¹, Met²-Ala¹, Met²-Ser¹, or Met²-Cys¹ at the N-terminus. These mutant proteins may also be expressed in *E. coli* by fusing a secretion signal peptide to the N-terminus. This signal peptide is cleaved from the polypeptide as part of the secretion process.

Yeast

Many strains of yeast cells known to those skilled in the art are also available as host cells for expression of the polypeptides of the present invention. Under another embodiment, the protein or fragment thereof of the present invention is expressed in a yeast cell, preferably *Saccharomyces cerevisiae*. The proteins or fragments thereof of the present invention can be expressed in *S. cerevisiae* by fusing it to the N-terminus of the URA3, CYC1 or ARG3 genes (Guarente and Ptashne, *Proc. Natl. Acad. Sci. (U.S.A.)* 78:2199-2203 (1981); Rose et al., *Proc. Natl. Acad. Sci. (U.S.A.)* 78:2460-2464 (1981); and Crabeel et al., *EMBO J.* 2:205-212 (1983)). Alternatively, proteins or fragments thereof of the present invention can be fused to either the PGK or TRP1 genes (Tuite et al., *EMBO J.* 1:603-608 (1982); and Dobson et al., *Nucleic Acids. Res.* 11:2287-2302 (1983)). More preferably, the protein or fragment thereof of the present invention is expressed as a mature protein (Hitzeman et al., *Nature* 293:717-722 (1981); Valenzuela et al., *Nature* 298:347-350 (1982); and Derynck et al., *Nucleic Acids Res.* 11:1819-1837 (1983)).

Native and engineered yeast promoters suitable for use in the present invention have been reviewed by Romanos et al., *Yeast* 8:423-488 (1992). Most preferably, the protein or fragment thereof of the present invention is secreted by the yeast cell (Blobel and Dobberstein, *J. Cell Biol.* 67:835-851 (1975); Kurjan and Herskowitz, *Cell* 30:933-943 (1982); Bostian et al., *Cell* 36:741-751 (1984); Rothman and Orci, *Nature* 355:409-415 (1992); Julius et al., *Cell* 32:839-852 (1983); and Julius et al., *Cell* 36:309-318 (1984)).

Mammalian

General methods for expression of foreign genes in mammalian cells have been reviewed (Kaufman, R. J., 1987, "Genetic Engineering, Principles and Methods", Vol. 9, J. K. Setlow, editor, Plenum Press, New York; Colosimo et al.,
5 *Biotechniques* 29: 314-331, 2000). Recombinant proteins are generally targeted to their natural locations within the host cell (e.g., cytoplasm, nucleus, or various membrane compartments), or are secreted, if a signal peptide is present. An expression vector is constructed in which a strong promoter capable of functioning in mammalian cells drives transcription of a eukaryotic secretion signal peptide
10 coding region, which is translationally joined to the coding region for the desired protein. For example, plasmids such as pcDNA I/Neo, pRc/RSV, and pRc/CMV (obtained from Invitrogen Corp., San Diego, California) can be used. The eukaryotic secretion signal peptide coding region can be from the gene itself or it can be from another secreted mammalian protein (Bayne, M. L. et al., *Proc. Natl. Acad. Sci. USA* 84: 2638-2642, 1987). After construction of the vector containing the gene, the vector DNA is transfected into mammalian cells such as the COS7,
15 HeLa, BHK, Chinese hamster ovary (CHO), or mouse L lines. The cells can be cultured, for example, in DMEM media (JRH Scientific). The polypeptide secreted into the media can be recovered by standard biochemical approaches following transient expression for 24 - 72 hours after transfection of the cells or after
20 establishment of stable cell lines following selection for antibiotic resistance. The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. See, e.g., Gething and Sambrook, *Nature*, 293:620-625, 1981, or alternatively, Kaufman et al, *Mol. Cell. Biol.*, 5(7):1750-1759, 1985) or Howley et al., and U.S. Pat. No. 4,419,446. Other suitable mammalian cell lines are the monkey COS-1 cell line and the CV-1 cell line.

Mammalian cells can also be used to express the nucleic acid molecules of the present invention. The nucleic acid molecules of the present invention can be
30 cloned into a suitable retroviral vector (see, e.g., Dunbar et al., *Blood* 85:3048-3057 (1995); Baum et al., *J. Hematother.* 5: 323-329 (1996); Bregni et al., *Blood* 80:1418-1422 (1992); Boris-Lawrie and Temin, *Curr. Opin. Genet. Dev.* 3:102-109 (1993); Boris-Lawrie and Temin, *Annal. New York Acad. Sci.* 716:59-71 (1994); Miller, *Current Top. Microbiol. Immunol.* 158:1-24 (1992)), adenovirus vector (Berkner, *BioTechniques* 6:616-629 (1988); Berkner, *Current Top. Microbiol. Immunol.* 158:39-66 (1992); Brody and Crystal, *Annal. New York Acad. Sci.* 716:90-103
35 (1994); Baldwin et al., *Gene Ther.* 4:1142-1149 (1997)), RSV, MuSV, SSV, MuLV

(Baum *et al.*, *J. Hematother.* 5: 323-329 (1996)), AAV (Chen *et al.*, *Gene Ther.* 5:50-58 (1998); Hallek *et al.*, *Cytokines Mol. Ther.* 2: 69-79 (1996)), AEV, AMV, or CMV (Griffiths *et al.*, *Biochem. J.* 241: 313-324 (1987)).

Transformation and transfection

5 In another aspect, the invention provides a transformed cell having a nucleic acid molecule which comprises an exogenous promoter region which functions in a cell to cause the production of an mRNA molecule which is linked to a structural nucleic acid molecule, wherein the structural nucleic acid molecule encodes an 11 alpha hydroxylase or oxidoreductase gene or fragment thereof. This
10 nucleic acid molecule is linked to a 3' non-translated sequence that functions in a cell to cause termination of transcription and addition of polyadenylated ribonucleotides to a 3' end of the mRNA molecule.

 Methods and compositions for transforming eukaryotic cells, bacteria and other microorganisms are known in the art (*see*, for example, Sambrook *et al.*,
15 *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1989); Colosimo *et al.*, *Biotechniques* 29: 314-331, 2000).

 Technology for introduction of DNA into cells is well known to those of skill in the art. Four general methods for delivering a gene into cells have been
20 described: (1) chemical methods (Graham and van der Eb, *Virology* 54:536-539 (1973)); (2) physical methods such as microinjection (Capecchi, *Cell* 22:479-488 (1980)), electroporation (Wong and Neumann, *Biochem. Biophys. Res. Commun.* 107:584-587 (1982); Fromm *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 82:5824-5828 (1985); U.S. Patent No. 5,384,253); and the gene gun (Johnston and Tang, *Methods*
25 *Cell Biol.* 43:353-365 (1994); (3) viral vectors (Clapp, *Clin. Perinatol.* 20:155-168 (1993); Lu *et al.*, *J. Exp. Med.* 178:2089-2096 (1993); Eglitis and Anderson, *Biotechniques*, 6:608-614 (1988)); and (4) receptor-mediated mechanisms (Curiel *et al.*, *Hum. Gen. Ther.* 3:147-154 (1992), Wagner *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 89:6099-6103 (1992)). Other methods well known in the art can also be used.

30 Transformation can be achieved using methods based on calcium phosphate precipitation, polyethylene glycol treatment, electroporation, and combinations of these treatments (*see* for example Potrykus *et al.*, *Mol. Gen. Genet.* 205:193-200 (1986); Lorz *et al.*, *Mol. Gen. Genet.* 199:178 (1985); Fromm *et al.*, *Nature* 319:791 (1986); Uchimiya *et al.*, *Mol. Gen. Genet.* 204:204 (1986); Marcotte
35 *et al.*, *Nature* 335:454-457 (1988)).

Assays for gene expression based on the transient expression of cloned nucleic acid constructs have been developed by introducing the nucleic acid molecules into cells by polyethylene glycol treatment, electroporation, or particle bombardment (Marcotte *et al.*, *Nature* 335: 454-457 (1988); McCarty *et al.*, *Cell* 66: 895-905 (1991); Hattori *et al.*, *Genes Dev.* 6: 609-618 (1992); Goff *et al.*, *EMBO J.* 9: 2517-2522 (1990)). Transient expression systems may be used to functionally dissect the regulatory and structural features of expression cassettes comprising operably-linked genetic elements.

Insect Cell Expression

Insect cells may be used as host cells to express recombinant proteins of the present invention (See, e.g., Luckow, V.A., *Protein Eng.* J. L. Cleland., Wiley-Liss, New York, NY: 183-218, 1996, and references cited therein). General methods for expression of foreign genes in insect cells using baculovirus vectors have been described (O'Reilly, D.R., L.K. Miller *et al.* *Baculovirus Expression Vectors: A Laboratory Manual*. New York, W.H. Freeman and Company, 1992; and King, L.A. and R.D. Possee, *The Baculovirus Expression System: A Laboratory Guide*, London, Chapman & Hall).

A baculovirus expression vector can be constructed by inserting the desired gene (e.g., 11 alpha hydroxylase or oxidoreductase) into a baculovirus transfer vector which can recombine into the baculovirus genome by homologous recombination. Many transfer vectors use a strong baculovirus promoter (such as the polyhedrin promoter) to drive transcription of the desired gene. Some vectors permit the expression of fusion proteins or direct the secretion of proteins from the cell by fusing a eukaryotic secretion signal peptide coding region to the coding region of the desired gene. The plasmid pVL1393 (obtained from Invitrogen Corp., San Diego, California) can be used, for example, to direct transcription of nonfused foreign genes in baculovirus-infected insect cells. The baculovirus transfer vector containing the desired gene is transfected into *Spodoptera frugiperda* (Sf9) insect cells along with circular or linearized genomic baculovirus DNA, and recombinant baculoviruses purified and amplified after one or more plaque assays.

Recombinant baculoviruses can also be created using the baculovirus shuttle vector system (Luckow, V.A. *et al.*, *J. Virol.* 67(8): 4566-4579, 1993; U.S. Patent 5,348,886) now marketed as the Bac-To-Bac™ Expression System (Life Technologies, Inc., Rockville, MD). The desired genes are inserted downstream from the polyhedrin promoter in mini-Tn7 cassettes that are transposed *in vivo*

into a baculovirus shuttle vector genome propagated in *E. coli*. Composite viral DNAs are isolated from *E. coli* and transfected into Sf9 cells and stocks of recombinant baculoviruses are rapidly prepared without the need for multiple rounds of tedious plaque purification common to methods that rely on homologous recombination.

Recombinant baculoviruses can also be created using the Gateway Recombinational Cloning System (Life Technologies) of shuttling genes from vector to vector using modified genetic elements (attachment sites) and modified proteins (e.g., int, IHF, xis) that are involved in the site-specific integration and excision of bacteriophage lambda.

Pure recombinant baculoviruses carrying the 11 alpha hydroxylase or oxidoreductase gene are used to infect cells cultured, for example, in Excell 401 serum-free medium (JRH Biosciences, Lenexa, Kansas) or Sf900-II (Life Technologies). Hydroxylases or oxidoreductases that are localized to membranes can be prepared using standard protocols that fractionate and enrich for enzymes in mitochondrial or microsomal fractions (Engel and White, *Dev Biol.* 140: 196-208, 1990). Hydroxylases or oxidoreductases that are secreted or leak into the medium can also be recovered by standard biochemical approaches.

Simultaneous expression of two or more recombinant proteins in baculovirus-infected insect cells can be carried out by two general approaches. The simplest approach is to coinfect insect cells with titrated stocks of recombinant baculoviruses harboring a single heterologous gene under the control of a strong baculovirus promoter, such as the polyhedrin or the p10 promoter. These promoters are highly transcribed during the late stages of infection when most host cell protein synthesis has been shut down. Earlier baculovirus promoters or other insect or eukaryotic cell promoters can also be used to direct synthesis at other times, which generally result in lower expression levels. Varying the ratio of two or more recombinant viruses used in a coinfection or selecting viruses that use different promoters to drive expression of the recombinant protein will permit one skilled in the art to select conditions suitable for optimal expression of the desired recombinant proteins.

Construction of dual- or multiple-expression vectors will also permit the expression of two or more recombinant proteins in baculovirus-infected insect cells. Generally, these vectors permit the introduction two or more gene cassettes into a single locus in the baculovirus genome. The structures of a variety of dual

expression vectors have been described (O'Reilly, D. R., L. K. Miller et al. *Baculovirus Expression Vectors: A Laboratory Manual*. New York, W.H. Freeman and Company, 1992; and King, L. A. and R. D. Possee, *The Baculovirus Expression System: A Laboratory Guide*, London, Chapman & Hall).

5 **Materials and Methods**

General methods

General methods of cloning, expressing, and characterizing proteins are found in T. Maniatis, et al., *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory, 1982, and references cited therein, incorporated herein by reference; and in J. Sambrook, et al., *Molecular Cloning, A Laboratory Manual*, 2nd edition, Cold Spring Harbor Laboratory, 1989, and references cited therein, incorporated herein by reference. General features and maps of a wide variety of cloning and expression vectors have been also been published (Gacesa, P. and Ramji, D.P., *Vectors: Essential Data*, John Wiley & Sons, 1994). General methods for the cloning and expression of genes in mammalian cells are also found in Colosimo et al., *Biotechniques* 29: 314-331, 2000. General and specific conditions and procedures for the construction, manipulation and isolation of polyclonal and monoclonal antibodies are well known in the art (See, for example, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, New York, 1988).

Unless noted otherwise, all specialty chemicals were obtained from Sigma (St. Louis, MO). Restriction endonucleases and T4 DNA ligase were obtained from Life Technologies (Rockville, MD), New England Biolabs (Beverly, MA), Roche Molecular Biochemicals (Indianapolis, IN), or Promega (Madison, WI). All parts are by weight and temperatures are in degrees centigrade (°C), unless otherwise indicated.

Strains, plasmids, and sequence cross listings

The bacterial strains used in these studies are listed in Table 1. Plasmids used or constructed for this study are listed in Table 2. Brief descriptions of sequences of relevant oligonucleotides, genes, or proteins are listed in Table 3.

Table 1: Strains

Designation	Description or Genotype	Reference/Source
DH5 α TM	F', <i>phi80 dlacZdeltaM15</i> , <i>delta(lacZYA-argF)U169</i> , <i>deoR</i> , <i>recA1</i> , <i>endA1</i> , <i>hsdR17</i> (rk',mk'), <i>phoA</i> , <i>supE44</i> , <i>lambda-</i> , <i>thi-1</i> , <i>gyrA96</i> , <i>relA1</i>	Life Technologies, Rockville, Maryland
DH10B TM	F, <i>mcrA</i> D(<i>mrr-hsdRMS-mcrBC</i>) <i>phi80 dlacZDM15 DlacX74 endA1</i> <i>recA1 deoR</i> D(<i>ara, leu</i>)7697 <i>araD139</i> <i>galU galK nupG rpsL</i>	Life Technologies, Rockville, Maryland
DH10Bac TM	DH10B harboring the baculovirus shuttle vector bMON14272 (Kan ^R) and the helper plasmid pMON7124 (Tet ^R)	Life Technologies, Rockville, Maryland; See also Luckow et al., <i>J.</i> <i>Viro.</i> 67: 4566-4579 (1993)

Table 2: Plasmids

Plasmid	SEQ ID NO.	Marker	Description	Source
pFastBac1		Amp ^R Gent ^R	Baculovirus donor plasmid containing multiple cloning site downstream of an AcNPV polyhedrin promoter within a mini-Tn7 transposable element capable of being transposed to a baculovirus shuttle vector	Life Technologies Inc. (Rockville, MD); See also Luckow et al., J. Virol. 67: 4566-4579 (1993)
pBluescript II SK		Amp ^R	Multifunctional phagemid cloning vector derived from pUC19.	Stratagene, La Jolla, CA
pCRII-TOPO		Amp ^R Kan ^R	Multifunctional cloning vector for direct cloning of polymerase chain reaction products using the T overhang	Invitrogen, Carlsbad, CA
pSport1		Amp ^R	Multifunctional cloning vector for cloning and in vitro transcription from either strand using SP6 or T7 promoters	Life Technologies, Rockville, MD
pGEM-T		Amp ^R	A derivative of pGEM-5Zf(+) with single 5' T overhangs at the insertion site to improve the efficiency of PCR product ligation	Promega, Madison, WI
pMON45624	#1	Amp ^R Gent ^R	pFastBac1 <i>EcoRI/XbaI</i> + PCR fragment <i>EcoRI/XbaI</i> encoding <i>Aspergillus ochraceus</i> 11 alpha hydroxylase	This work

pMON45603		Amp ^R	pBluescriptII SK <i>Bam</i> HI/ <i>Hinc</i> II + <i>Bam</i> HI/ <i>Hinc</i> II 5' segment of human oxidoreductase	This work
pMON45604		Amp ^R	pBluescriptII SK <i>Hinc</i> II/ <i>Kpn</i> I + <i>Hinc</i> II/ <i>Kpn</i> I 3' segment of human oxidoreductase	This work
pMON45605	#3	Amp ^R Gent ^R	pFastBac1 <i>Bam</i> HI/ <i>Kpn</i> I + <i>Bam</i> HI/ <i>Kpn</i> I complete coding region of human oxidoreductase cDNA.	This work
pMON45630		Amp ^R Kan ^R	pCRII-TOPO <i>Sal</i> I/ <i>Bam</i> HI + <i>Sal</i> I/ <i>Bam</i> HI 5' segment of <i>A.</i> <i>ochraceus</i> oxidoreductase cDNA	This work
pMON45631		Amp ^R Kan ^R	pCRII-TOPO <i>Bam</i> HI/ <i>Xho</i> I + <i>Bam</i> HI/ <i>Xho</i> I 3' segment of <i>A.</i> <i>ochraceus</i> oxidoreductase cDNA which lacked the intron.	This work
pMON45632	#5	Amp ^R Gent ^R	pFastBac1 <i>Sal</i> I/ <i>Xho</i> I + containing assembled coding region of <i>Aspergillus</i> <i>ochraceus</i> oxidoreductase	This work

Table 3: Table of Sequences

SEQ ID NO	Description	Length/Sequence	Type
(SEQ ID NO: 01)	Nucleotide sequence of <i>Aspergillus ochraceus</i> 11alphaOH gene from pMON45624	1776	DNA
(SEQ ID NO: 02)	<i>Aspergillus ochraceus</i> 11alphaOH protein sequence from pMON45624	514	Protein
(SEQ ID NO: 03)	Nucleotide sequence of human oxidoreductase gene from pMON45605	2031	DNA
(SEQ ID NO: 04)	Human oxidoreductase protein sequence from pMON45605	677	Protein
(SEQ ID NO: 05)	Nucleotide sequence of <i>Aspergillus ochraceus</i> oxidoreductase gene from pMON45632	2322	DNA
(SEQ ID NO: 06)	<i>Aspergillus ochraceus</i> oxidoreductase protein sequence from pMON45632	705	Protein
(SEQ ID NO: 07)	Primer H. oxred 1A	gatcggatccaatATGG GAGACTCCACGTGGAC AC	DNA
(SEQ ID NO: 08)	Primer H. oxred 1B	CAGCTGGTTGACGAGAG CAGAG	DNA
(SEQ ID NO: 09)	Primer H. oxred 2A	CTCTGCTCTCGTCAACC AGCTG	DNA
(SEQ ID NO: 10)	Primer H. oxred 2B	gatcgggtacctaGCTC CACACGTCCAGGGAGTA G	DNA
(SEQ ID NO: 11)	Primer A.oxred-for1	GACGGIGCIGGTACAAT GGA	DNA
(SEQ ID NO: 12)	Primer A.oxred-rev1	TTAIGACCAIACATCIT CCTGGTAGC	DNA
(SEQ ID NO: 13)	Primer pSport-for1	CAAGCTCTAATACGACT CACTATAGGGA	DNA
(SEQ ID NO: 14)	Primer A.oxred-rev2	CAGGAACCGATCGACCT CGGAA	DNA
(SEQ ID NO: 15)	Primer A.oxred-rev3	GTCACCCCTCACCAGCAG AGCCAATG	DNA
(SEQ ID NO: 16)	Primer A.oxred-rev4	CCACATTGCGAACCATA GCGTTGTAGTG	DNA
(SEQ ID NO: 17)	Primer pSport-for2	GCCAAGCTCTAATACGA CTCACTATAGGGAAAGC	DNA
(SEQ ID NO: 18)	Primer A.oxred-for2	gtcgacATGGCGCAACT CGATACTCTC	DNA
(SEQ ID NO: 19)	Primer A.oxred-rev5	ctcgagttaGGACCAGA CATCGTCCTGGTAG	DNA
(SEQ ID NO: 20)	Primer A.oxred-for3	GGATCCCTCGCGACCTG TGATCAT	DNA
(SEQ ID NO: 21)	Primer A.oxred-for4	CGAAGATTTCTTGACA AGGATGAATGGAAGACT TTTC	DNA
(SEQ ID NO: 22)	Primer A.oxred-rev6	CTGAAAAGTCTTCCATT CATCCTTGTACAAGAAA TC	DNA
(SEQ ID NO: 23)	11aOH peptide 1	AAAYWLATLQPSDLPEL N	Protein
(SEQ ID NO: 24)	11aOH peptide 2	CRQILTPYIHKRKS LKG TTDE	Protein
(SEQ ID NO: 25)	11aOH peptide 3	HMGFGHGVHACPRFFA	Protein

(SEQ ID NO: 26)	oxr peptide 1	SNEI CTYWAVAKDPYASAGPA MNG	Protein
(SEQ ID NO: 27)	CAA75565; cytochrome P450 monooxygenase [<i>Gibberella</i> <i>fujikuroi</i>]		Protein
(SEQ ID NO: 28)	CAB91316; probable cytochrome P450 monooxygenase (lovA) [<i>Neurospora crassa</i>]		Protein
(SEQ ID NO: 29)	CAB56503; cytochrome P450 [<i>Catharanthus roseus</i>]		Protein
(SEQ ID NO: 30)	AAB94588; CYP71D10p [<i>Glycine</i> <i>max</i>]		Protein
(SEQ ID NO: 31)	CAA75566; cytochrome P450 monooxygenase [<i>Gibberella</i> <i>fujikuroi</i>]		Protein
(SEQ ID NO: 32)	AAD34552; cytochrome P450 monooxygenase [<i>Aspergillus</i> <i>terreus</i>]		Protein
(SEQ ID NO: 33)	CAA75567; cytochrome P450 monooxygenase [<i>Gibberella</i> <i>fujikuroi</i>]		Protein
(SEQ ID NO: 34)	CAA76703; cytochrome P450 [<i>Gibberella fujikuroi</i>]		Protein
(SEQ ID NO: 35)	CAA57874; unnamed protein product [<i>Fusarium oxysporum</i>]		Protein
(SEQ ID NO: 36)	CAA91268; similar to cytochrome P450-cDNA EST yk423b11.3 comes from this gene; [<i>Caenorhabditis elegans</i>]		Protein
(SEQ ID NO: 37)	BAA02936 NADPH-cytochrome P450 reductase precursor [<i>Saccharomyces cerevisiae</i>]		Protein
(SEQ ID NO: 38)	CAA81550 NADPH cytochrome P450 oxidoreductase [<i>Aspergillus</i> <i>niger</i>]		Protein
(SEQ ID NO: 39)	BAA04496 NADPH-cytochrome P450 oxidoreductase [<i>Mus musculus</i>]		Protein
(SEQ ID NO: 40)	Universal bacteriophage M13 reverse primer	CAG GAA ACA GCT ATG AC	DNA
(SEQ ID NO: 41)	Universal bacteriophage T7 promoter primer	TAA TAC GAC TCA CTA TAG GG	DNA
(SEQ ID NO: 42)	<i>Aspergillus ochraceus</i> Primer 11alphaOH-for	gatcgaattcATGCCCT TCTTCACTGGGCT	DNA
(SEQ ID NO: 43)	<i>Aspergillus ochraceus</i> Primer 11alphaOH-rev	gatctctagattacaca gttaaactcgccaTATC GAT	DNA
(SEQ ID NO: 44)	pFastBac1 Primer Bacfwd	CTGTTTTCGTAACAGTT TTG	DNA
(SEQ ID NO: 45)	pFastBac1 Primer PolyA	CCTCTACAAATGTGGTA TG	DNA
(SEQ ID NO: 46)	<i>Aspergillus ochraceus</i> Primer 45624-for1	GAGATCAAGATTGCCTT	DNA
(SEQ ID NO: 47)	<i>Aspergillus ochraceus</i> Primer 45624-for2	CTTCGACGCTCTCAA	DNA
(SEQ ID NO: 48)	<i>Aspergillus ochraceus</i> Primer 45624-rev1	GCAATCTTGATCTCGTT	DNA
(SEQ ID NO: 49)	S90469 human cytochrome P450 reductase [placental, mRNA Partial, 2403 nt].	2403	DNA
(SEQ ID NO: 50)	AAB21814 human cytochrome P450 reductase, placental, partial	676	Protein
(SEQ ID NO: 51)	A60557 human NADPH- ferrihemoprotein reductase	677	Protein
(SEQ ID NO: 52)	P16435 Human NADPH-cytochrome P450 reductase	677	Protein

(SEQ ID NO: 53)	P00389 Rabbit NADPH-cytochrome P450 reductase	679	Protein
(SEQ ID NO: 54)	P00388 Rat NADPH-cytochrome P450 reductase	678	Protein
(SEQ ID NO: 55)	P37040 Mouse NADPH-cytochrome P450 reductase	678	Protein
(SEQ ID NO: 56)	P04175 Pig NADPH-cytochrome P450 reductase	678	Protein
(SEQ ID NO: 57)	Universal bacteriophage SP6 primer	gatttaggtgacactat ag	DNA
(SEQ ID NO: 58)	NotI-poly-dT adapter	5' - pGACTAGT TCTAGA TCGCGA GCGGCCGC CC (T) ₁₂ - 3'	DNA
(SEQ ID NO: 59)	SalI adapter, top strand	5' - TCGACCCACGCGTCCG - 3'	DNA
(SEQ ID NO: 60)	SalI adapter, bottom strand	3' - GGGTGCGCAGGCp - 5'	DNA
(SEQ ID NO: 61)	Primer oxred 1C	GTGGACCACAAGCTCGT ACTG	DNA
(SEQ ID NO: 62)	Primer oxred 2C	CATCGACCACCTGTGTG AGCTG	DNA
(SEQ ID NO: 63)	Primer oxred 2D	GTACAGGTAGTCCTCAT CCGAG	DNA
(SEQ ID NO: 64)	<i>Aspergillus niger</i> NADP CYP450 oxidoreductase Z26838	3710	DNA
(SEQ ID NO: 65)	<i>Aspergillus niger</i> NADP CYP450 oxidoreductase CAA81550	693	Protein

Specific Methods

Transformation of E. coli strains

E. coli strains such as DH5 alpha and DH10B (Life Technologies, Rockville, MD) are routinely used for transformation of ligation reactions and are the hosts used to prepare plasmid DNA for transfecting mammalian cells. *E. coli* strains, such as DH10B and MON105 (Obukowicz, et al., *Appl. and Envir. Micr.*, 58: 1511-1523, 1992) can be used for expressing the proteins of the present invention in the cytoplasm or periplasmic space.

DH10B and DH5alpha subcloning efficiency cells are purchased as competent cells and are ready for transformation using the manufacturer's protocol. Other *E. coli* strains are rendered competent to take up DNA using a CaCl₂ method. Typically, 20 to 50 mL of cells are grown in LB medium (1% Bacto-tryptone, 0.5% Bacto-yeast extract, 150 mM NaCl) to a density of approximately 1.0 absorbance unit at 600 nanometers (OD600) as measured by a Baush & Lomb Spectronic spectrophotometer (Rochester, NY). The cells are collected by

centrifugation and resuspended in one-fifth culture volume of CaCl_2 solution [50 mM CaCl_2 , 10 mM Tris-Cl ((10 mM 2-amino-2-(hydroxymethyl) 1,3-propanediol hydrochloride, pH 7.4] and are held at 4°C for 30 minutes. The cells are again collected by centrifugation and resuspended in one-tenth culture volume of CaCl_2 solution. Ligated DNA is added to 0.1 ml of these cells, and the samples are held at 4°C for 30-60 minutes. The samples are shifted to 42°C for 45 seconds and 1.0 ml of LB is added prior to shaking the samples at 37°C for one hour. Cells from these samples are spread on plates (LB medium plus 1.5% Bacto-agar) containing either ampicillin (100 micrograms/mL, ug/ml) when selecting for ampicillin-resistant transformants, or spectinomycin (75 ug/ml) when selecting for spectinomycin-resistant transformants. The plates are incubated overnight at 37°C. Colonies are picked and inoculated into LB plus appropriate antibiotic (100 ug/ml ampicillin or 75 ug/ml spectinomycin) and are grown at 37°C while shaking.

DNA isolation and characterization

Plasmid DNA can be isolated by a number of different methods and using commercially available kits known to those skilled in the art. Plasmid DNA is isolated using the Promega Wizard™ Miniprep kit (Madison, WI), the Qiagen QIAwell Plasmid isolation kits (Chatsworth, CA) or Qiagen Plasmid Midi or Mini kit. These kits follow the same general procedure for plasmid DNA isolation. Briefly, cells are pelleted by centrifugation (5000 x g), the plasmid DNA released with sequential NaOH/acid treatment, and cellular debris is removed by centrifugation (10000 x g). The supernatant (containing the plasmid DNA) is loaded onto a column containing a DNA-binding resin, the column is washed, and plasmid DNA eluted. After screening for the colonies with the plasmid of interest, the *E. coli* cells are inoculated into 50-100 ml of LB plus appropriate antibiotic for overnight growth at 37°C in an air incubator while shaking. The purified plasmid DNA is used for DNA sequencing, further restriction enzyme digestion, additional subcloning of DNA fragments and transfection into *E. coli*, mammalian cells, or other cell types.

DNA Sequencing protocols

Purified plasmid DNA is resuspended in dH_2O and its concentration is determined by measuring the absorbance at 260/280 nm in a Baush and Lomb Spectronic 601 UV spectrometer. DNA samples are sequenced using ABI PRISM™ DyeDeoxy™ terminator sequencing chemistry (Applied Biosystems Division of Perkin Elmer Corporation, Lincoln City, CA) kits (Part Number 401388 or 402078)

according to the manufacturer's suggested protocol. Occasionally, 5% DMSO is added to the mixture in repeat experiments, to facilitate the sequencing of difficult templates.

Sequencing reactions are performed in a DNA thermal cycler (Perkin
5 Elmer Corporation, Norwalk, CT) following the recommended amplification conditions. Typically, DNA samples were prepared containing 500 ng of template DNA and 100 ng of primer of choice in thin-walled 0.2 mL PCR tubes that have been brought to 12 uL with Millipore milli-Q (mQ)-quality water. 2 ul of 2 mM Mg⁺⁺ was added to each tube. Tubes were denatured for 5 minutes at 96°C in a
10 Perkin-Elmer System 9700 thermal cycler. After denaturation, the tubes were chilled to a temperature of 4°C by the thermal cycler. 6 ul of ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit was added to each tube. The samples were returned to the thermal cycler and cycle-sequenced using the following program: (1) 96°C for 30 sec; (2) 50°C for 5 sec; (3) 60°C for 4 min,
15 followed by step (1) for 24 additional cycles and then held at 4°C. Cycle sequencing was complete after about 2.5 hours.

Samples are purified to remove excess dye terminators with using Centri-SepTM spin columns (Princeton Separations, Adelphia, NJ) or purified through a
20 Millipore MAHV N45 50 Multiscreen-HV filtration plate which had been filled with 25 uL Sephadex G-50 superfine resin and 300 uL mQ water. Before loading samples onto filtration plates, the plate was prespun in a centrifuge at 750 x g for 2 min to remove excess water. The samples were loaded onto the resin and the plate spun again at 750 x g for 4 min. The purified sample was collected into a 96-well plate that was placed directly underneath the Sephadex-filled plate during the
25 spin. The liquid in the 96-well plate was dried at room temperature in a Speed Vac. After 45-60 min the DNA was dried and pelleted at the bottom of the plate. Samples were resuspended in 3 uL of a formamide/blue Dextran loading dye and were heated for 2 minutes (see p.33 of Perkin-Elmer Big Dye manual for loading buffer recipe). Samples were loaded onto 48 cm well-to-read length 4.5%
30 acrylamide gels and sequenced for 7 hr using ABI automated DNA sequencers (typically run module Seq Run 48E-1200 and dye set DT, Program BD, Set Any-Primer).

Overlapping DNA sequence fragments are analyzed and assembled into master DNA contigs using Sequencher DNA Analysis software (Gene Codes
35 Corporation, Ann Arbor, MI) or the Perkin-Elmer Data Collection and Sequence Analysis programs to assign bases to the data collected.

BLAST, ClustalW, and Boxshade homology alignment tools

A variety of programs can be used to align nucleotide or peptide sequences to each other and to facilitate homology searches in large sequence databases. BLAST (Basic Local Alignment Search Tool), which implements the statistical matching theory by Karlin and Altschul (*Proc. Natl. Acad. Sci. USA* 87: 2264-2268, 1990; *Proc. Natl. Acad. Sci. USA* 90: 5873-5877, 1993), is a widely used program for rapidly detecting ungapped nucleotide or peptide subsequences that match a given query sequence (Available from the National Center for Biotechnology Information, <http://www.ncbi.nlm.nih.gov>). BLAST uses a heuristic algorithm which seeks local as opposed to global alignments and is therefore able to detect relationships among sequences which share only isolated regions of similarity (Altschul et al., *J. Mol. Biol.* 215: 403-410, 1990).

Two parameters can be varied which alter the sensitivity and quantity of BLAST search results. Parameter B (with a default value of 10) regulates the number of high-scoring segment pairs (alignments) reported in the results. Parameter V (with a default value of 10) is the maximum number of database sequences (hits) for which one-line descriptions will be reported. Matches are based on high-scoring segment pairs (HSPs). Two sequences may share more than one HSP, if the HSPs are separated by gaps. The BLAST algorithm is sensitive to ambiguities in the sequence and is not well-suited for sequences that contain many gaps.

The program *blastp* compares an amino acid query sequence against a protein sequence database. *blastn* compares a nucleotide query sequence against a nucleotide sequence database. *blastx* compares a nucleotide query sequence translated in all reading frames against a protein sequence database. You could use this option to find potential translation products of an unknown nucleotide sequence. *tblastn* compares a protein query sequence against a nucleotide sequence database dynamically translated in all reading frames. *tblastx* compares the six-frame translations of a nucleotide query sequence against the six-frame translations of a nucleotide sequence database (See <http://www.ncbi.nlm.nih.gov/Education/BLASTinfo/> for more information on BLAST, related programs, and pattern matching algorithms).

Nucleotides searches performed with BLAST, score = 98-557, word length 514 letters, were used to obtain nucleotide sequences homologous to nucleic acid molecules of the present invention. Protein searches are performed with BLASTP,

score = 50, word length = 3 to obtain amino acid sequences homologous to a reference polypeptide (e.g., SEQ ID NO: 2).

5 Clustal W version 1.74, which implements a different algorithm for alignment of multiple DNA or protein sequences, was also used to prepare alignments and to assign percent identities between different sequences. This program improves the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice (Thompson et al., *Nucleic Acids Research*, 22(22):4673-4680, 1994). The default parameters for version 1.74 were used facilitate alignments and to assign percent identities between two sequences. The input consisted of sequences in FASTA format and the output is the alignment shown in the figures. For nucleic acid sequences, the iub DNA weight matrix was used. For amino acid sequences, the blosum protein weight matrix was used (See <http://www.ncbi.nlm.nih.gov/Education/BLASTinfo/> for more information on BLAST, related programs, and pattern matching algorithms.

Boxshade v 3.31 is a public domain program for creating nicely formatted printouts from multiple-aligned protein or DNA sequences. Boxshade, by itself, does not create alignments, but applies shading or coloring to files that were previously prepared by other sequence alignment programs. The inputs to Boxshade are the alignments created by ClustalW and the threshold values for the residues to be colored or shaded. In most cases, except where specified, a 50% identity value was used. With this setting, if a position has greater than or equal to half of the sequences sharing an identical residue, then it is shaded. Boxshade is available by ftp from ftp. or by e-mail from Kay Hofmann (khofmann@isrec-sun1-unil.ch or Michael D. Baron (michael.baron@bbsrc.ac.uk).

Protein Purification and Characterization

Protein purification can be accomplished using any of a variety of chromatographic methods such as: ion exchange, gel filtration or hydrophobic chromatography or reversed phase HPLC. In some cases, proteins which are properly folded can be affinity-purified using affinity reagents, such as monoclonal antibodies or receptor subunits attached to a suitable matrix. These and other protein purification methods are described in detail in *Methods in Enzymology*, Volume 182 "Guide to Protein Purification" edited by Murray Deutscher, Academic Press, San Diego, California, 1990.

The purified protein can be analyzed by RP-HPLC, electrospray mass spectrometry, and SDS-PAGE. Protein quantitation is done by amino acid composition, RP-HPLC, and/or Bradford protein dye-binding assays. In some cases, tryptic peptide mapping is performed in conjunction with electrospray mass spectrometry to confirm the identity of the protein.

Examples

The following examples will illustrate the invention in greater detail, although it will be understood that the invention is not limited to these specific examples. Various other examples will be apparent to the person skilled in the art after reading the present disclosure without departing from the spirit and scope of the invention. It is intended that all such other examples be included within the scope of the appended claims.

Example 1 - Preparation of *A. ochraceus* spores for RNA extraction

Aspergillus ochraceus ATCC 18500 stock culture (50 ul) was grown for 3-4 days on plates containing sporulation medium: 50 g/L molasses, 5 g/L cornsteep liquid, 5 g/L KH_2PO_4 , 25 g/L NaCl, 25 g/L glucose, 20 g/L agar, and 0.4 g/L progesterone, pH 5.8. Progesterone was included in the media to induce the steroid 11 α -hydroxylase. Spores were scraped from the plates into 5 to 7 ml saline, washed in saline, collected by centrifugation, and suspended in saline containing 15% glycerol. The spores were frozen on dry ice and stored at -80°C. Approximately 0.8 g spores were incubated at 30°C in a 1 liter flask containing 400 ml 1% glucose, 50 mM KH_2PO_4 and 0.1 g canrenone, pH 7.0. This treatment prior to spore disruption has three benefits: (1) to induce the steroid 11 α -hydroxylase by incubation with canrenone; (2) to determine whether the spores were catalyzing the 11 α -hydroxylation of canrenone; (3) and to soften the spore wall. After approximately 26 hours of incubating with shaking at 30°C to provide better aeration, the spores were collected by centrifugation. Visual inspection with the aid of a microscope indicated that very few had started to germinate. The spore pellets were flash frozen in liquid nitrogen and stored at -80°C. The media was analyzed for presence of 11 alpha hydroxy canrenone by HPLC to determine whether spores used for library construction demonstrated the desired activity.

Example 2 - *A. ochraceus* spores catalyze 11 α -hydroxylation of canrenone

Approximately 160 ml of media from the spore induction was extracted three times with 70 ml ethyl acetate to collect the steroid substrate and products. The organic phase was dried over anhydrous magnesium sulfate, filtered, and evaporated to dryness. The residue was dissolved in 8 ml methanol so that the final concentration of canrenone was approximately 15 mM (assuming quantitative recovery). The media extract was diluted 10- to 15-fold into 50% methanol for HPLC analysis. Stock solutions of canrenone and 11 α -hydroxy canrenone were prepared in methanol. Standards for HPLC analysis were prepared from these stock solutions by diluting to a final concentration of 750 μ M with 50% methanol. Media extract and standards were chromatographed over a C-4 reverse phase HPLC column. The media exhibited a component with the same retention time as the 11 α -hydroxy canrenone standard, as monitored at 254 nm (data not shown).

Example 3 - Growth of *A. ochraceus* Mycelia for RNA extraction

Liquid cultures of *Aspergillus ochraceus* mycelia were grown in 10 g/L peptone, 10 g/L yeast extract and 10 g/L glucose containing 20 g/L canrenone for 24 to 72 hours at 28°C in a volume of 160 ml. Ten ml samples of cells were filtered, washed with cold water, frozen, and stored at -80°C.

Example 4 - Extraction of total RNA from induced spores

Approximately 0.4 g spores were disrupted in 40 ml Trizol reagent (Life Technologies, Rockville, MD) using a Mini-Beadbeater™ model 3110 (Biospec Products, Bartlesville, OK). Briefly, spore-Trizol mixture was subjected to four 30 second pulses at low speed. Between pulses, tubes containing spores were chilled on ice. Visual inspection with the aid of a microscope indicated that the majority of the spores were disrupted by this treatment. The debris was pelleted by low-speed centrifugation and the total RNA in the supernatant was extracted following the manufacturer's recommended protocols for use with Trizol. Briefly, 2 ml chloroform was added for each 10 ml Trizol in 11 ml polypropylene centrifuge tubes. Following a 3 minute extraction of proteins, phase separation was done by centrifugation and the aqueous phase containing the RNA was transferred to a clean tube for precipitation with an equal volume of isopropanol. The precipitated RNA was recovered by centrifugation and washed with 70% ethanol. The RNA was resuspended in 10 ml water, re-extracted with chloroform and precipitated with ethanol overnight at -20°C. Total RNA (3 mg) was recovered by

centrifugation and rehydrated in 2 ml water, and precipitated on ice by adding an equal volume of cold 4 M lithium chloride. This precipitation was done to remove DNA, carbohydrates, heme, and other impurities which can carry over from guanidine methods. The RNA was recovered by a 25 minute centrifugation.

5 **Example 5 - Extraction of total RNA from induced mycelia**

Approximately 0.5 g wet weight cells were pulverized to a fine powder under liquid nitrogen with a mortar and pestle pre-chilled in dry ice. The powder was added to 10 ml Trizol Reagent (Life Technologies) and homogenized with a Kinematica polytron (Kinematica AG, Lucerne, Switzerland) at setting #4.
10 Cellular debris was removed by centrifugation prior to chloroform extraction. The aqueous phase containing nucleic acids was precipitated with isopropanol for 10 minutes at room temperature. The precipitate was collected by centrifugation and washed with 70% ethanol. The RNA was rehydrated in water and re-extracted with chloroform to remove any residual proteins. The aqueous phase was
15 precipitated at -20°C with 1/10 volume of 3 M sodium acetate and 2.5 volumes absolute ethanol. The final yield was 424 ug. Approximately 4 ug and 16 ug of total RNA were separated by electrophoresis through a 1.2% agarose gel and visualized by staining in ethidium bromide. Chromosomal DNA was present as a minor contaminant.

20 **Example 6 - Extraction of Total RNA from HepG2 cells**

Hepatocellular human liver carcinoma cells (HepG2), ATCC HB-8065, were maintained in DMEM high glucose media supplemented with Penstrep, glutamate and 10% fetal bovine serum (Life Technologies, Rockville, MD). Cells were induced overnight with 0.05% ethanol and harvested for RNA extraction by trypsinization.
25 Briefly, the cell pellet was resuspended in >10X volumes of 4 M guanidine isothiocyanate, 50 mM Tris-HCl, pH 7.5, 25 mM EDTA (solution D, Life Technologies) and then vortexed. Water and sodium acetate, pH 4.1, were added such that the final concentration of sodium acetate was 0.1 M. The RNA solution was extracted with one half volume of chloroform and placed on ice for 15 minutes.
30 The aqueous phase was re-extracted with chloroform and precipitated overnight with isopropanol. Total RNA was resuspended in solution D and re-precipitated with isopropanol, followed by two precipitations in water containing 0.3 M sodium acetate pH 5.5 and 2.5 volumes of ethanol. PolyA⁺ selection was performed twice as described below.

Example 7 - PolyA⁺ Selection of mRNA

PolyA⁺ RNA was selected from total RNA with an Eppendorf 5Prime, Inc. kit (Boulder CO). Briefly, each 1 mg of total RNA was selected twice over a column containing oligo dT cellulose. The column slurry was packed by gentle centrifugation and equilibrated with 0.5 M NaCl. RNA was allowed to bind to the dT cellulose for 15 minutes at room temperature. The columns were washed once with 0.5 M NaCl, and twice with 0.1 M NaCl. PolyA⁺ RNA was eluted in 0.5 ml 10 mM Tris-HCl, 1 mM EDTA, pH 7.5. The selection by oligo dT cellulose was performed twice. The mRNA was precipitated at -20°C with 0.3 M sodium acetate in 50% ethanol, with glycogen added as carrier.

Example 8 - cDNA Synthesis and Library Construction

The Superscript™ Plasmid System for cDNA Synthesis and Plasmid Cloning kit (Life Technologies) was used for cDNA synthesis and library construction. Superscript II reverse transcriptase catalyzed the first strand of cDNA in a 20 ul reaction for 1 hour at 42°C. The final composition was 50 mM Tris-HCl, pH 8.3, 75 mM KCl, 3 mM MgCl₂, 10 mM DTT, 50 uM each dATP, dCTP, dGTP and dTTP, 50 ug/ml oligo-dT-NotI primer-adaptors that were phosphorylated at their 5' end (Life Technologies) and 50,000 units/ml Superscript II reverse transcriptase.

oligo-dT-NotI primer-adapter

5' - pGACTAGT TCTAGA TCGCGA GCGGCCGC CC (T)₁₂ - 3' (SEQ ID NO: 58)

SpeI XbaI NruI NotI

A radiolabeled tracer ([α-³²P]dCTP) was not added. The second strand of cDNA was synthesized in a reaction volume of 150 ul. The final composition of this mixture including the first strand reaction was 25 mM Tris-HCl, pH 7.5, 100 mM KCl, 5 mM (NH₄)₂SO₄, 0.15 mM B-NAD⁺, 250 uM each dATP, dCTP, dGTP and dTTP, 1.2 mM DTT, 65 units/ml *E. coli* DNA ligase, 250 units/ml *E. coli* DNA polymerase I and 13 units/ml *E. coli* Rnase H. After a 2 hour incubation at 16°C, 10 units of T4 DNA polymerase was added, and incubated 5 minutes at 16°C. The reaction was stopped with 10 ul 0.5 M EDTA and the cDNA was separated from cDNAs smaller than 300 base pairs, primer-adaptors and deoxynucleotides with GENECLAN II (BIO 101 Inc. La Jolla, CA). Annealed *Sal* I adaptors (Life Technologies) that were phosphorylated at their 5' blunt end were ligated to the cDNA overnight at 16°C.

*Sal*I adapter

5' - TCGACCCACGCGTCCG - 3' (SEQ ID NO: 59)
3' - GGGTGCGCAGGCP - 5' (SEQ ID NO: 60)

GENECLEAN II was used to remove the adaptors. The cDNA was then digested with *NotI*. QIAquick columns (QIAGEN, Valencia, CA) were used to remove small DNA fragments from the cDNA, which was ethanol precipitated.

Example 9 - Size Fractionation of cDNA

The cDNA was enriched for species approximately 1.5 kb and larger by gel electrophoresis through 0.8% Sea-Plaque agarose (FMC BioProducts, Rockland ME) in TAE buffer. The preparative gel had a lane of DNA size markers which was excised from the gel after electrophoresis and stained with ethidium bromide for visualization under ultraviolet light next to a ruler so that the appropriate region of the cDNA could be recovered from the gel. GENECLEAN II was used to extract the cDNA, which was eluted in 20 ul water.

Example 10 - Library Construction in Vector pSport1 and Electroporation into *E. coli*

An aliquot of the size-selected cDNA was ligated overnight at 4°C with pSport1 (Life Technologies, Inc., Rockville, MD) predigested with *NotI* and *SalI* in a 20 ul reaction containing 50 mM Tris-HCl, pH 7.6, 10 mM MgCl₂, 1 mM ATP, 5% (w/v) PEG 8000, 1 mM DTT, 2.5 ug/ml pSport1, approximately 0.5 ug/ml cDNA, and 50 units/ml T4 DNA ligase. The ligation mixture was precipitated by the addition of 12.5 ul 7.5 M ammonium acetate, 5 ul yeast tRNA carrier and 70 ul absolute ethanol. The ligated cDNA was recovered by centrifugation at room temperature for 20 minutes and rehydrated in 5 ul sterile water. One ul of the ligated cDNA was introduced into ElectroMAX DH10B *E. coli* (Life Technologies) by electroporation. Cells were allowed to recover in 1 ml SOC medium (Life Technologies) for 1 hour at 37°C, before plating an aliquot on LB with 100 ug/ml ampicillin. The titer of the *Aspergillus ochraceus* spore library (designated LIB3025) was determined by preparing serial dilutions of the cell suspension in SOC. The equivalent of 1 ul, 0.1 ul and 0.01 ul samples of the cell suspension were plated, and the resulting titer was calculated to be 1.75 x 10⁶/ml colony forming units.

**Example 11 - Identification of clones encoding cytochrome P450 enzymes
by DNA sequence analysis and construction of plasmid pMON45624
encoding *Aspergillus ochraceus* 11 alpha hydroxylase**

Cloning of 11 alpha hydroxylase from Aspergillus ochraceus

5 Approximately 2,000 colonies were selected on LB agar plates containing
100 ug/ml ampicillin and miniprep plasmid DNA samples were prepared for
sequencing. Unidirectional sequencing was performed from the 3' end of the
expressed sequence tags (ESTs) beginning at the *NotI* site encompassing part of
the poly dT primer used for cDNA synthesis. Two universal primers were used to
10 facilitate the sequencing:

M13 reverse: CAG GAA ACA GCT ATG AC (SEQ ID NO: 40)
T7 promoter: TAA TAC GAC TCA CTA TAG GG (SEQ ID NO: 41)

15 Most known cytochrome p450s contain a conserved heme-binding region
approximately 50 amino acid residues (150 nucleotides) upstream of the stop codon
(Nelson *et al*, *Pharmacogenetics* 6: 1-42, 1996). The 2,000 ESTs were screened for
sequences encoding the canonical heme-binding motif (FXXGXXXCXG, where "X"
is any amino acid) in the appropriate region using BLASTX and visual inspection
of the sequences scored as hydroxylases for the canonical heme-binding motif.
20 Only fifteen ESTs had the heme-binding motif. One EST was unique and the other
fourteen appeared to be overlapping sequences. The cDNA inserts from seven
clones encoding putative cytochrome p450 enzymes were then sequenced to
completion. All seven encoded the same enzyme.

Gene Amplification of Aspergillus ochraceus 11 alpha hydroxylase

25 The coding region of the 11 alpha hydroxylase was amplified by PCR using
a unique clone from the *A. ochraceus* cDNA spore library (LIB3025) as a template.
The primers included recognition sites for *EcoRI* (forward) and *XbaI* (reverse) for
directional cloning into pFastbac1. Amplification was carried out for 32 cycles
using a PCR core kit (Roche) and 50 pmol of each primer. One cycle consisted of a
30 denaturation step at 94°C for 45 seconds, an annealing step at 60°C for 45 seconds,
and an elongation step at 72°C for 60 seconds.

Primer 11alphaOH-for: gatcgaattcatGCCCTTCTTCACTGGGCT (SEQ ID NO: 42)
Primer 11alphaOH-rev: gatctctagattACACAGTTAAACTCGCCATATCGAT (SEQ ID NO: 43)

Construction of pMON45624

The amplified fragments described above were purified through a QIAquick column (Qiagen, Valencia CA) and digested with *EcoRI* and *XbaI* prior to ligation into pFastBac1 cleaved with *EcoRI* and *XbaI*. The resulting plasmid was designated pMON45624 and the DNA sequence verified using primers based on the vector sequence and internal primers based on the 11 alpha hydroxylase sequence (shown below).

Primer Bacfwd: CTGTTTTCGTAACAGTTTGT (SEQ ID NO: 44)
 Primer PolyA: CCTCTACAAATGTGGTATG (SEQ ID NO: 45)
 Primer 45624-for1: GAGATCAAGATTGCCTT (SEQ ID NO: 46)
 Primer 45624-for2: CTTCGACGCTCTCAA (SEQ ID NO: 47)
 Primer 45624-rev1: GCAATCTTGATCTCGTT (SEQ ID NO: 48)

The nucleotide and predicted amino acid sequences of the cloned 11 alpha hydroxylase are displayed in Figure 1 as SEQ ID NO: 1 and SEQ ID NO: 2, respectively.

Figure 4 sets forth an amino acid homology alignment of *A. ochraceus* 11 alpha hydroxylase cloned in pMON45624 and aligned with related enzymes found in GenBank using BLAST. Figure 5 is a phylogenetic tree showing the this relationship graphically. Figure 6 shows the percent homology between *Aspergillus ochraceus* steroid 11 alpha hydroxylase and the top 10 enzymes found in GenBank using BLAST, calculated using Clustal W and Boxshade.

Example 12 - Amplification of cDNA encoding human NADPH Cytochrome P450 reductase and cloning into plasmids pMON45603, pMON45604, and pMON45605

Gene Amplification of human oxidoreductase

Approximately 1 ug polyA⁺ mRNA from HepG2 cells was heated to 65°C for 10 minutes with 100 ng random hexamers (Invitrogen, Carlsbad, CA) in an 11 ul reaction. The mixture was chilled on ice, then incubated at 42°C for 75 minutes in a 20 ul reaction containing 1 ul RNase inhibitor (Promega, Madison, WI), 0.01 M DTT, 5 mM dNTPs, 50 mM Tris-HCl, pH 8.3, 75 mM KCl, 3 mM MgCl₂, and 1 ul SuperScriptII enzyme (Life Technologies). The reverse transcriptase was inactivated by heating to 95°C for 2 minutes. First strand cDNA was stored at -20°C. Forward and reverse primers were based on the nucleotide sequence of accession number S90469 (human placental partial mRNA encoding cytochrome P450 reductase (SEQ ID NO: 49)). The accession number of the corresponding

protein sequence is AAB21814 (SEQ ID NO: 50). The human oxidoreductase was cloned in two pieces which were assembled in pFastBac1 (Life Technologies) by ligation at an internal *HincII* site. The primers included restriction sites for directional subcloning into pFastBac1.

5

Primer H. oxred 1A: gatcggatccaatATGGGAGACTCCCACGTGGACAC (SEQ ID NO: 07)
 Primer H. oxred 1B: CAGCTGGTTGACGAGAGCAGAG (SEQ ID NO: 08)
 Primer H. oxred 2A: CTCTGCTCTCGTCAACCAGCTG (SEQ ID NO: 09)
 Primer H. oxred 2B: gatcggtagcttaGCTCCACACGTCCAGGGAGTAG (SEQ ID NO: 10)

10 The second strand was synthesized using 400 uM dNTP and 167 nM of each primer set per 150 ul reaction. Amplification was performed with Deep Vent polymerase (New England Biolabs, Beverly, MA). The reaction for segment 2 (the 3' half of the oxidoreductase cDNA) was adjusted to 5% DMSO. The amplification included an initial cycle of denaturation at 94°C for 90 seconds, followed by annealing at 62°C
 15 for 2 minutes and elongation at 72°C for 2 minutes. This was followed by 30 cycles, consisting of a 45 second denaturation step, a 45 second annealing step, and a 60 second elongation step. The elongation step was extended to 5 minutes for the final cycle.

Construction of pMON45603, pMON45604, pMON45605

20 The PCR fragments for the 5' half of the oxidoreductase cDNA were digested with *Bam*HI and *Hinc*II. The PCR fragments for the 3' half of the oxidoreductase cDNA were digested with *Hinc*II and *Kpn*I and ligated into pBluescript II (Stratagene, La Jolla, CA) for sequencing. The resulting plasmids were designated pMON45603 (5' segment) and pMON45604 (3' segment). The
 25 *Bam*HI/*Hinc*II fragment from pMON45603 and the *Hinc*II/*Kpn*I fragment from pMON45604 were ligated into pFastbac1 cut with *Bam*HI and *Kpn*I, to generate pMON45605.

Sequencing primers were based on the sequence of GenBank accession number S90469 (SEQ ID NO 49), a cDNA encoding cytochrome P450 reductase
 30 [human, placenta, mRNA Partial, 2403 nt]. The cognate protein sequence is: AAB21814 (SEQ ID NO 50) cytochrome P450 reductase {EC 1.6.2.4} [human, placenta, Peptide Partial, 676 aa] [Homo sapiens]. The cDNA insert of pMON45603 was sequenced using primer oxred 1C, and the cDNA insert of pMON45604 was sequenced using primer oxred 2C and 2D. Universal T7 (SEQ ID
 35 NO: 41) and M13 reverse (SEQ ID NO: 40) primers, which annealed to vector sequences flanking the cDNA inserts were also used for sequencing.

Primer oxred 1C: GTGGACCACAAGCTCGTACTG (SEQ ID NO: 61)

Primer oxred 2C: CATCGACCACCTGTGTGAGCTG (SEQ ID NO: 62)

Primer oxred 2D: GTACAGCTAGTCTCATCCGAG (SEQ ID NO: 63)

The nucleotide and predicted amino acid sequences of the cloned human oxidoreductase are displayed in Figure 2 as SEQ ID NO: 3 and SEQ ID NO: 4, respectively. Figure 11 sets forth an alignment of human oxidoreductase with top 4 hits from SwissProt. Figure 12 sets forth a phylogenetic tree displaying the genetic relatedness of human oxidoreductase, to these hits. Figure 13 shows the percent identity between human oxidoreductase and top 4 hits from SwissProt.

Example 13 - Amplification of cDNA encoding NADPH cytochrome P450 reductase from *A. ochraceus* and cloning into plasmids pMON45630, pMON45631, and pMON45632.

Gene Amplification of Aspergillus ochraceus oxidoreductase

An alignment of sequences from the *Aspergillus niger* cprA gene accession number Z26938 (SEQ ID NO: 65) and a partial cDNA clone 804561639F1 from *Aspergillus fumigatus* (PathoSeq Database, Incyte Pharmaceuticals) was visually scanned to select regions of high homology for the design of primers for PCR. A primer set was selected which spanned the coding region of the cprA gene product from amino acids 203 to 693.

Primers were selected from the 5' most region of overlap where the amino acid sequence was identical between both and the nucleic acid sequence differed by 2 positions in the 3rd codon position. For the 3' primer, the nucleic acid encoding the stop codon, last 7 amino acid residues and 2 additional bases corresponding to second and third positions in the codon of the amino acid residue 8 positions from the stop codon encodes ARG in *A. niger* and SER in *A. fumigatis* (CGC vs. AGC). Inosines replaced the third base in codons when there was a discrepancy between the *A. niger* and *A. fumigatus* sequence.

Primer A.oxred-for1: GACGGIGCIGGTACAATGGA (SEQ ID NO: 11)

Primer A.oxred-rev1: TTAIGACCAIACATCITCCTGGTAGC (SEQ ID NO: 12)

(where I = Inosine)

A partial cDNA clone was amplified from approximately 5 ug of total RNA extracted from *A. ochraceus* mycelia. Before the first strand synthesis, the RNA was heated to 65°C for 10 minutes with 100 ng random hexamers (Promega Madison WI) in an 11 ul reaction mixture. The mixture was chilled on ice, then incubated at 42°C for 75 minutes in a 20 ul reaction containing 1 ul RNase inhibitor (Promega), 0.01 M DTT, 5 mM dNTPs, 50 mM Tris-HCl, pH 8.3, 75 mM

KCl, 3 mM MgCl₂ and 1 ul SuperScriptII (LTI). The reverse transcriptase was inactivated by heating to 95°C for 2 minutes. The first strand cDNA was stored at -20°C. The second strand was synthesized using 5 ul of the first strand as template. The reaction included 500 nM primers, 200 uM each dNTP, and Taq polymerase and buffer as supplied in PCR core kit (Roche Molecular Biochemicals, Indianapolis, IN). Amplification was performed using 32 cycles of a 30 second denaturation step at 94°C, a 30 second annealing step at 60°C and a 60 second elongation step at 72°C. The amplified DNA products were cloned into pGEM-T (Promega, Madison, WI) and sequenced using universal T7 (SEQ ID NO: 41) and SP6 (SEQ ID NO: 57) primers.

Primer SP6 GATTAGGTGACACTATAG (SEQ ID NO: 57)

Alignment of the sequences with the *A. niger* cprA gene revealed that the *A. ochraceus* clones had an intron in the same position as the intron in the *A. niger* gene. This indicated that the *A. ochraceus* PCR products might have been amplified from a genomic DNA contaminant of the total RNA. A reverse primer based on the *A. ochraceus* sequence was designed to amplify the approximately 600 missing bp including the initial methionine. The *A. ochraceus* cDNA library was then used as a template for PCR. The forward primer was based on the reverse complement of vector pSport1 (Life Technologies) bases 299 to 326. The other primer, A.oxred-rev2 was based on the *A. ochraceus* sequence encoding residues 326-333.

Primer pSport-for1: CAAGCTCTAATACGACTCACTATAGGGA (SEQ ID NO: 13)

Primer A.oxred-rev2: CAGGAACCGATCGACCTCGGAA (SEQ ID NO: 14)

The *A. ochraceus* spore library size made from gel-purified fragments >1.5 kb in size was then used as a template for amplifying the final 200 bases of coding region. Two new reverse primers were designed from the A.oxred sequence, and a new forward primer based on pSport1 (bases 295-328) was also used.

Primer A.oxred-rev3: GTCACCCTCACCAGCAGAGCCAATG (SEQ ID NO: 15)

Primer A.oxred-rev4: CCACATGCGAACCATAGCGTTGTAGTG (SEQ ID NO: 16)

Primer pSport-for2: GCCAAGCTCTAATACGACTCACTATAGGAAAGC (SEQ ID NO: 17)

Amplification was performed using an Elongase polymerase kit (Life Technologies, Rockville MD) for 35 cycles consisting of a denaturation step at 94°C for 30 seconds, an annealing step at 63°C for 30 seconds, and an elongation step at 68°C for 5 minutes. The PCR products were cloned directly into pCRII TOPO (Invitrogen). Twelve clones were sequenced, and the composite sequence, extended

for 232 bases upstream of the initial methionine, and included 2 in-frame stop codons (Data not shown).

Primers incorporating the complete coding region of A.oxred were designed with a 5' *SaI*I site and a 3' *Xho*I site for ligation into expression vector pFastBac1.

5

Primer A.oxred-for2: gtcgacATGGCGCAACTCGATACTCTC (SEQ ID NO: 18)

Primer A.oxred-rev5: ctcgagttaGGACCAGACATCGTCCTGGTAG (SEQ ID NO: 19)

A. *ochraceus* total RNA was used as a template for PCR with these primers and the Elongase kit. Amplification consisted of 35 cycles with a 30 second denaturation step at 94°C, a 30 second annealing step at 64°C, and a 5 minute elongation step at 68°C. An aliquot of the cDNA from reaction ran as a single band of approximately 2.1 kb.

10

Construction of pMON45630

The PCR products were cloned directly into pCRII-TOPO (Invitrogen, Carlsbad, CA). All clones contained the internal intron noted earlier. One clone was designated pMON45630.

15

Construction of pMON45631 and pMON45632

A strategy based on two step PCR from an internal *Bam*HI site approximately 170 bp upstream of the 5' splice site was employed to generate clones lacking the intron.

20

Primer A.oxred-for3: GGATCCCTCGCGACCTGTGATCAT (SEQ ID NO: 20)

Primer A.oxred-for4: CGAAGATTCTTGTACAAGGATGAATGGAAGACTTTTC (SEQ ID NO: 21)

Primer A.oxred-rev6: CTGAAAAGTCTCCATTCATCCTTGTACAAGAAATC (SEQ ID NO: 22)

Primers A.oxred-for4 and rev6 were complementary and flanked the intron. The first PCR reaction used an A.oxred clone linearized at the internal *Bam*HI site as template. Polymerase and buffers were supplied by the PCR core kit (Roche Molecular Biochemicals, Indianapolis, IN). Primer and dNTP concentrations were 500 nM and 200 uM, respectively. Two reactions were performed, using a combination of A.oxred-for3 with A.oxred-rev6, and A.oxred-for4 with A.oxred-rev5. Following a 2 minute initial denaturation, 28 cycles of PCR amplification were performed. One cycle included a 45 second denaturation at 94°C, a 45 second denaturation step at 62°C and a 45 second elongation step at 72°C. One ul of each reaction served as template for the second PCR amplification with primers A.oxred-for3 and A.oxred-rev5 using Elongase enzyme and buffers. Amplification

25

30

35

consisted of 30 cycles with a 30 second denaturation step at 94°C, a 30 second annealing step at 62°C, and a 5 minute elongation step at 68°C. The PCR products were directly cloned into pCRII-TOPO. DNA sequencing demonstrated that the intron had been removed. This clone was designated pMON45631.

5 Plasmid pMON45632 was constructed in a three-way ligation by ligating the *SalI/BamHI* fragment from pMON45630 with the *BamHI/XhoI* fragment from pMON45631 and vector pFastBac1, which had been cut with *SalI* and *XhoI* and dephosphorylated to enhance the recovery of vectors with the desired inserts.

10 The nucleotide and amino acid sequences of the cloned *Aspergillus ochraceus* 11 oxidoreductase are displayed in Figure 3 as SEQ ID NO: 5 and SEQ ID NO: 6, respectively. Figure 7 sets forth the amino acid homology of *Aspergillus ochraceus* and human oxidoreductase to NADPH cytochrome P450 reductases from *A. niger*, mouse, and *S. cerevisiae*. Figure 8 sets forth the amino acid alignment for *A. ochraceus*, *A. niger*, and *S. cerevisiae* oxidoreductases. Figure 9 is a phylogenetic tree showing the relatedness of *Aspergillus ochraceus* and human oxidoreductase to reductases from *A. niger*, yeast, and mouse. Figure 10 shows the percent homology between *Aspergillus ochraceus* steroid 11 alpha hydroxylase and the oxidoreductases from *A. niger*, yeast, and mouse, calculated using Clustal W and Boxshade.

20 **Example 15: Generation of polyclonal antibodies recognizing *Aspergillus ochraceus* 11 alpha hydroxylase and *Aspergillus ochraceus* NADPH cytochrome p450 reductase**

Generation of anti-11-a-hydroxylase Antibodies

25 Polyclonal antibodies against *Aspergillus ochraceus* 11 alpha hydroxylase and NADPH cytochrome p450 reductase were raised in rabbits against synthetic peptides (prepared by Sigma/Genosis, The Woodlands, TX) corresponding to several regions of the following predicted protein sequences:

30 11aOH peptide 1: AAAYWLATLQPSDLPELN (SEQ ID NO: 23)
 11aOH peptide 2: CRQILTPYIHKRSLKGTDT (SEQ ID NO: 24)
 11aOH peptide 3: HMGFGHGVHACPGRRFFASNEI (SEQ ID NO: 25)
 oxr peptide 1: CTYWAVAKDPYASAGFAMNG (SEQ ID NO: 26)

35 The 11aOH peptide 2 (SEQ ID NO: 24) corresponds to the G helix, G/H loop, and H helix region present in an alignment of the amino acid sequence of 11 alpha hydroxylase with the corresponding sequence of CYP3A4 described by Wang and

Lu, (*Drug Metab. Dispos.* 25(6), 762-767, 1997). The 11aOH peptide 3 (SEQ ID NO: 25) corresponded to the peptide fragment from the heme-binding domain.

Immunological grade peptides were monitored for purity using reverse phase high performance liquid chromatography (HPLC). Each peptide was conjugated to keyhole limpet hemacyanin (KLH) and suspended in Complete Freund's Adjuvant. The conjugated peptide was then injected subcutaneously at multiple sites into rabbits. Each conjugated peptide was injected into two rabbits. All subsequent immunizations were given in incomplete Freund's Adjuvant. In general, five subsequent injections were given at two-week intervals following the initial immunization. IgG fractions were affinity-purified using a Sepharose-Protein A column. Fractions from the two rabbits injected with each peptide were combined at a 1:1 ratio. The pooled anti-11 alpha hydroxylase (rabbits GN 1187/1188) was 0.34 mg/ml IgG. The pooled anti-oxred (rabbits GN 2023/2024) was 0.26 mg/ml IgG. The combined IgGs were each diluted 1:10, 1:100 and 1:1,000 for a pilot experiment to determine which was dilution was optimal for probing Western blots. The 1:10 dilution gave the best results and was used for probing subsequent Westerns.

Example 16 - Insect Cell Infection and Heterologous Expression

Proteins were expressed in Sf9 insect cells using baculovirus shuttle vectors (Luckow et al., *J. Virol.* 67: 4566-4579, 1993). The baculovirus shuttle vector (bacmid) contains a mini-F replicon for expression in bacterial cells, a kanamycin resistance marker for selection, and attTn7 (the target site for the bacterial Tn7 transposon) within the *lacZα* sequence. Each of these elements is inserted into the polyhedrin locus of the *Autographa californica* nuclear polyhedrosis virus (AcNPV, the native baculovirus) genome. A donor plasmid (pFastBac1, Life Technologies) was used to deliver the gene to be expressed and was inserted into the bacmid via the bacterial Tn7 transposition elements. pFastBac1 contains the Tn7 left and right ends flanking the polyhedrin promoter, a polylinker cloning sequence, the SV40 polyA transcription termination sequence, and the gentamicin resistance gene for selection. Recombinant viruses were generated following transformation of the pFastBac1 plasmid, which contained a single 11 alpha hydroxylase or oxidoreductase cDNA, into DH10Bac *E. coli* cells (Life Technologies) that contained the bacmid and helper plasmid.

Transfections were performed using CellFectin™ reagent (Life Technologies) following the manufacturer's protocol for *Spodoptera frugiperda*

(Sf9) cells. Cells were seeded in 6-well tissue culture plates at 9×10^5 cells per well in SF-900 serum-free medium (Life Technologies) and allowed to attach for at least one hour. The transfection mixtures were made following the addition of 5 μ l miniprep DNA and 5 μ l Cellfectin to polystyrene tubes that contained 200 μ l SF-900 medium. The mixtures were allowed to incubate for 15-30 minutes at room temperature. Prior to transfection, 800 μ l SF-900 medium was added to each tube. The cells were washed one time with 2 ml SF-900 medium, and the DNA mixtures were added to the cells. The cultures were allowed to incubate for 5 hours at 27°C. Following the 5 hr incubation period, the transfection mixture was removed and the cultures were replenished with 3 ml per well IPL-41 medium (Life Technologies) supplemented with 10% fetal bovine serum. Following a three day incubation period, the cells were harvested, centrifuged, and the supernatant that contained recombinant virus (designated as passage 1 or P1 stock) was removed and stored at 4°C. A larger viral stock was made by infecting 100 ml fresh Sf9 cells at 5×10^5 cells per ml with 0.5 ml of the P1 medium. This larger (P2) stock was then titered using a plaque assay protocol (O'Reilly et al., 1992), and used for production of the 11 alpha hydroxylase or oxidoreductase enzymes, separately or in combination with each other.

Figure 14 sets forth an immunoblot illustrating expression of *Aspergillus ochraceus* P450 11 alpha hydroxylase in baculovirus-infected insect cells harvested at 25 and 48 hours post infection. The nitrocellulose membrane was probed with a 1:1 mixture of antibodies prepared two rabbits immunized with a conjugated synthetic peptide 11aOH peptide 2 (SEQ ID NO 24).

Figure 15 sets forth an immunoblot illustrating expression of *Aspergillus ochraceus* P450 oxidoreductase in baculovirus-infected insect cells harvested at 25 and 48 hours post infection. The nitrocellulose membrane was probed with a 1:1 mixture of antibodies prepared two rabbits immunized with a conjugated synthetic peptide oxr peptide 1 (SEQ ID NO 26).

Example 17: Co-infection baculoviruses expressing of *Aspergillus ochraceus* 11 alpha hydroxylase and human oxidoreductase

Sf9 cells were co-infected with virus particles that contained the steroid 11 alpha hydroxylase cDNA and a separate virus containing a human NADPH P450-oxidoreductase. Both viruses were added at a multiplicity of infection (MOI) ratio of 0.1 : 0.01 (11 aOH to oxr). One day after infection, 0.9 μ g/ml hemin chloride was added to the culture. The cells were harvested by centrifugation three days after

infection (unless specified differently), and the washed cell pellets were frozen until processed for sub-cellular fractions.

Example 18: Co-infection baculoviruses expressing of *Aspergillus ochraceus* 11 alpha hydroxylase and *Aspergillus ochraceus* oxidoreductase

Sf9 cells are co-infected with virus particles that contain the steroid 11 alpha hydroxylase cDNA and a separate virus containing *A. ochraceus* NADPH P450-oxidoreductase. Both viruses are added at a multiplicity of infection (MOI) ratio of 0.1 : 0.01 (11 aOH to oxr). One day after infection, 0.9 µg/ml hemin chloride is added to the culture. The cells are harvested by centrifugation three days after infection (unless specified differently), and the washed cell pellets are frozen until needed in subsequent experiments that require processing into for sub-cellular fractions.

Example 19: Preparation of subcellular fractions from baculovirus-infected insect cells

One half gram of the cell pastes from infected sf9 cells and uninfected control cells were thawed and suspended in 40 ml of 0.25 M sucrose with 10 mM KHPO₄, adjusted to pH 7.4. The suspensions were homogenized using a Fisher Sonic Dismembrator, model 300 probe sonicator (Fisher Scientific, St. Louis, MO). The samples were transferred to a conical centrifuge tube (Corning Costar Corporation, Cambridge, MA) and subjected to centrifugation at 500 x g at 5°C for 15 minutes. The pellets were resuspended in the same volume of fresh buffer and viewed under a microscope to confirm complete lysis. Few or no whole cells were observed. The supernatants were then subjected to centrifugation at 10,000 x g for 30 minutes at 5°C to collect mitochondria, Golgi and other subcellular organelles. The pellets were resuspended in fresh buffer and subjected to centrifugation at 7,800 x g for 30 minutes at 5°C to collect mitochondria.

The mitochondrial pellets were resuspended in buffer as described about and the centrifugation was repeated. The mitochondrial pellets were resuspended in 2 ml buffered sucrose solution and stored at -80°C in 100 µl aliquots.

The supernatants from the original mitochondrial fractionation were subjected to centrifugation at 200,000 x g for 1 hour at 5°C. The microsomal pellets were resuspended in 2 ml buffered sucrose solution and stored at -80°C in 100 µl aliquots.

Microsomal Incubations

Incubation mixtures consisted of Sf9 microsomes (1.0 mg of protein/mL final concentration), an NADPH-generating system and 250 μ M substrate (AD) in 100 mM potassium phosphate buffer, pH 7.4 or 150 mM HEPES buffer, pH 7.4. The NADPH-generating system was composed of the following at the indicated final concentrations: MgCl_2 (7.5 mM), D-glucose-6-phosphate (7.5 mM), NADP (0.80 mM), and glucose-6-phosphate dehydrogenase (1.0 units/mL). Incubations were carried out for the indicated times at 37°C in a water bath. Following incubation, reactions were terminated by the addition of 0.3 ml methanol. The samples were vortexed three times for two seconds and placed on ice, or stored at -70°C for later analysis.

Example 20: HPLC assays to measure conversion of steroid substrates to their hydroxylated counterparts

High Performance Liquid Chromatography (HPLC)

The HPLC method used to separate hydroxylated steroid compounds from steroid substrates, such as 11 α -hydroxyandrostenedione from androstenedione, is a modified version of the testosterone hydroxylase assay, described by Sonderfan et al., *Arch. Biochem. Biophys.* **255**: 27-41, 1987). The standards for androstenedione and 11-beta-hydroxyandrostenedione were obtained from Sigma. 11-alpha-hydroxyandrostenedione (89.5% pure, with the major impurity being androstenedione) was provided by Searle Medicinal Chemistry. HPLC grade water and methanol were obtained from Burdick & Jackson.

The HPLC system consisted of a Model 1050 series pump, autoinjector and variable wavelength detector (Hewlett-Packard, Naperville, IL), and a Model TC-50 temperature controller and Model CH-30 column heater (both Eppendorf, Madison, WI).

Cell membrane fractions derived from insect cells transfected with recombinant baculoviruses expressing 11-hydroxylase and complementary electron transport proteins were analyzed for 11-hydroxylase activity in a reaction mixture containing 80 mM phosphate buffer, pH 7.4, 8 mM MgCl_2 , and 0.9 mM NADP⁺ in a final volume of 200 μ l. In order to insure an adequate source of reducing equivalents, an NADPH regenerating system was provided by adding glucose-6-phosphate dehydrogenase (1.5 U/ml) and 8 mM glucose-6-phosphate. Steroid substrate (e.g., androstenedione) was provided at a final concentration of 0.3 mM.

Reaction mixtures were incubated at 37°C for 30 min. The reactions were terminated by the addition of 200 ul methanol and then placed on ice. Samples were pelleted by centrifugation to remove precipitated protein.

5 On one occasion, the incubation was carried out in a volume of 0.5 ml in siliconized polypropylene 1.5 ml microcentrifuge tubes at 37°C for 120 minutes. The enzyme, prepared from microsomal or mitochondrial fractions, was added and the substrate added at a concentration of 250 μ M (e.g., 25 mM methanol stock solution of AD). The cofactor buffer was 100 mM potassium phosphate, pH 7.4, 7.5 mM $MgCl_2$, 7.5 mM glucose-6-phosphate, 0.80 mM NADP, and 1.0 units/mL
10 glucose-6-phosphate dehydrogenase. HPLC samples were prepared by terminating the 0.5 ml reaction mixture by addition of 0.3 ml methanol, vortexing three times for 2 seconds and storing on ice. The tubes were spun for 5 minutes at $\sim 20,000 \times g$ in a microcentrifuge and the samples transferred to autosampler vials and capped.

15 Steroid components present in reaction mixtures and media extract were separated and analyzed by reverse phase HPLC using a 250 mm x 4 mm Vydac analytical C-4 column. Chromatograms were developed using a solvent gradient from 40% to 100% methanol over a ten minute time period and holding at 100% methanol for 5 minutes before re-equilibration to initial conditions. The column effluent was monitored for UV absorbance at both 254 and 220 nm.

20 Androstenedione, testosterone and monohydroxylated androstenedione metabolites were resolved on a Nova-pak C18 column, 4 micron, 3.9 x 150 mm (Waters Chromatography, Milford, MA) equipped with a 0.22 micron Rheodyne precolumn filter at 40°C and 1.0 ml mobile phase/min. A stepped gradient was utilized with water as mobile phase solvent A and methanol as solvent B. The
25 initial concentration of solvent B was 42% for 6 min. The percentage of B was increased linearly to 45% over 4 minutes and then held for 3 minutes. The percentage of B was then increased linearly to 80% over 10 minutes and held there for an additional 2 minutes for a total run time of 25 minutes. The ultraviolet detection wavelength was 247 nm and the injection volume was 200 ul.

30 Both the "mitochondria" sample and the "microsomal" sample produced peaks matching the HPLC retention time of the 11 α -hydroxyandrostenedione standard, while other fractions did not. These "mitochondria" and "microsomal" peaks were 3.2 and 2.3%, respectively, of the total peak area quantitated at 247 nm. The 11 α -hydroxyandrostenedione standard was also spiked into a blank
35 microsomal incubation sample at a concentration of 5.0 μ g/mL. The concentration

of the "mitochondria" and "microsomal" 11 α -hydroxyandrostenedione peaks were 1.75 and 1.31 μ g/mL, after correcting for the purity of the standard (89.5%). These concentrations represent 2.3 and 1.7% of substrate converted to 11 α -hydroxyandrostenedione, using a substrate concentration of 250 μ M.

5 Figure 16 sets forth an HPLC tracing illustrating the conversion of androstenedione (AD) to its 11 alpha hydroxy counterpart after incubating AD with subcellular fractions prepared from baculovirus-infected insect cells expressing *Aspergillus ochraceus* 11 alpha hydroxylase and human oxidoreductase.

10 **Example 21: Recognition of *Aspergillus ochraceus* 11 alpha hydroxylase and *Aspergillus ochraceus* NADPH cytochrome p450 reductase by immunoblotting using polyclonal antibodies generated against synthetic peptides**

 Proteins from Sf9 cell lysates (obtained from uninfected and recombinant baculovirus-infected cells) were loaded onto lanes of a 10% gradient acrylamide
15 mini gel (BioRad, Hercules, CA) at equal concentrations (10 μ g per well). The proteins were separated by electrophoresis at 16 mAmps constant current for approximately 1 hr in a Tris-glycine buffer containing 0.1% SDS (Sigma, St. Louis, MO). The proteins were transferred to nitrocellulose (Schleicher & Schuell, Keene, NH) for 40 min at 70 mAmps constant current. Primary antibodies were diluted
20 1:10 (from stock concentrations of 0.34 mg/ml IgG for anti-11 alpha hydroxylase (antibodies GN-1187 and GN-1188 prepared from peptide 11aOH peptide 2 CRQILTPYIHKRKSLSKGTDD (SEQ ID NO: 24)), and 0.26 mg/ml IgG for anti-oxred (antibodies GN-2023 and GN-12024 prepared from oxr peptide 1 CTYWAVAKDPYASAGPAMNG (SEQ ID NO: 26)) and used to probe the
25 nitrocellulose membrane. The antigens were detected using anti-rabbit horseradish peroxidase (HRP)-linked secondary antibody as recommended by the manufacturer (New England Biolabs, Beverly, MA). Chemiluminescence was detected using luminol and peroxide reagents (New England Biolabs, Beverly, MA) following the protocol provided by the vendor. Light emission was recorded using
30 X-OMAT AR film (Eastman Kodak Company, Rochester, NY). Images were recorded using a Minolta Dimage V digital camera (Minolta Corporation, Ramsey, NJ).

Example 22: Characterization of the *Aspergillus ochraceus* genomic DNA encoding 11 alpha hydroxylase and oxidoreductase

The approaches described above can be used to facilitate the identification of genes encoding steroid hydroxylases and oxidoreductases within the genome of *Aspergillus ochraceus* and closely related microorganisms, including *Aspergillus niger* and *Aspergillus nidulans*. Other preferred organisms are *Rhizopus oryzae*, *Rhizopus stolonifer*, *Streptomyces fradiae*, *Bacillus megaterium*, *Pseudomonas cruciviae*, *Trichothecium roseum*, *Fusarium oxysporum f. sp. cepae*, *Rhizopus arrhizus*, and *Monosporium olivaceum*. Other preferred organisms that are known to have steroid 11 alpha hydroxylase activity are described in the detailed description of the invention, above.

Briefly, genomic DNA is prepared and shotgun cloned into low copy artificial chromosomes propagated in bacteria. A large number of clones are sequenced to ensure statistical representation of the entire genome, and the sequences of overlapping clones merged to produce the final map and sequence of the genome. Analysis of the open reading frames, will reveal regions which are homologous to the steroid hydroxylase and oxidoreductase genes of the present invention, and regions of the translated open reading frames which are homologous to these enzymes using programs designed to facilitate multiple sequence alignments of nucleotide and protein sequence data such as BLAST, CLUSTAL W, and BoxShade. Genes which encode these proteins are obtained from the artificial chromosomes and recloned into expression vectors such as pFastBac1, transformed into appropriate host cells, which are assayed for the presence of enzymes capable of carrying out the conversion of steroid substrates to their oxidized counterparts.

It is intended that the scope of the present invention be determined by reference to the appended claims. It is recognized that a number of variations can be made to this invention as it is currently described but which do not depart from the scope and spirit of the invention without compromising any of its advantages. These include isolation of homologous genes from microorganisms known to carry out 11 alpha hydroxylation of steroid substrates, preferably fungi and bacteria. This invention is also directed to any substitution of analogous components. This includes, but is not restricted to use of these techniques to isolate other P450s which are involved in steroidogenesis, including hydroxylases that act at other

positions in the core molecule, and use of these enzymes to facilitate bioconversion of steroid intermediates in modified host microorganisms.

All references, patents, or applications cited herein are incorporated by reference in their entirety, as if written herein.

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Claims

1. An isolated and purified nucleic acid, encoding *Aspergillus ochraceus* 11 alpha hydroxylase.
2. An isolated DNA, encoding *Aspergillus ochraceus* 11 alpha hydroxylase.
- 5 3. An isolated cDNA, encoding *Aspergillus ochraceus* 11 alpha hydroxylase.
4. An isolated gene, encoding *Aspergillus ochraceus* 11 alpha hydroxylase.
5. An isolated allele of the gene encoding *Aspergillus ochraceus* 11 alpha hydroxylase.
- 10 6. An isolated and purified nucleic acid, wherein said nucleic acid sequence is as set forth in SEQ ID NO: 1.
7. An isolated DNA, wherein said DNA sequence is as set forth in SEQ ID NO: 1.
8. An isolated cDNA, wherein said cDNA sequence is as set forth in SEQ ID NO: 1.
- 15 9. An isolated gene, wherein said gene sequence is as set forth in SEQ ID NO: 1.
10. An isolated allele of a gene, wherein said gene sequence is as set forth in SEQ ID NO: 1.
- 20 11. An isolated protein having the amino acid sequence of *Aspergillus ochraceus* 11 alpha hydroxylase.
12. An isolated variant of the protein having the amino acid sequence of *Aspergillus ochraceus* 11 alpha hydroxylase.
13. A fusion protein comprising the amino acid sequence of *Aspergillus*
25 *ochraceus* 11 alpha hydroxylase.
14. An isolated protein wherein the amino acid sequence of said protein is as set forth in SEQ ID NO: 2.

15. An isolated variant of a protein wherein the amino acid sequence of said protein is as set forth in SEQ ID NO: 2.
16. A purified polypeptide, the amino acid sequence of which comprises SEQ ID NO: 2 with at least one conservative amino acid substitution.
- 5 17. A purified polypeptide, the amino acid sequence of which comprises a sequence at least 99% identical to SEQ ID NO: 2.
18. A purified polypeptide, the amino acid sequence of which comprises a sequence at least 95% identical to SEQ ID NO: 2.
- 10 19. A purified polypeptide, the amino acid sequence of which comprises a sequence at least 90% identical to SEQ ID NO: 2.
20. A purified polypeptide, the amino acid sequence of which comprises a sequence at least 75% identical to SEQ ID NO: 2.
21. A purified polypeptide, the amino acid sequence of which comprises a sequence at least 50% identical to SEQ ID NO: 2.
- 15 22. An isolated and purified nucleic acid, encoding *Aspergillus ochraceus* 11 alpha oxidoreductase.
23. An isolated DNA, encoding *Aspergillus ochraceus* oxidoreductase.
24. An isolated cDNA, encoding *Aspergillus ochraceus* oxidoreductase.
- 20 25. An isolated gene, encoding *Aspergillus ochraceus* oxidoreductase.
26. An isolated allele of the gene encoding *Aspergillus ochraceus* oxidoreductase.
27. An isolated and purified nucleic acid, wherein said nucleic acid sequence is as set forth in SEQ ID NO: 5.
- 25 28. An isolated DNA, wherein said DNA sequence is as set forth in SEQ ID NO: 5.
29. An isolated cDNA, wherein said cDNA sequence is as set forth in SEQ ID NO: 5.

30. An isolated gene, wherein said gene sequence is as set forth in SEQ ID NO: 5.
31. An isolated allele of a gene, wherein said gene sequence is as set forth in SEQ ID NO: 5.
- 5
32. An isolated protein having the amino acid sequence of *Aspergillus ochraceus* oxidoreductase.
33. An isolated variant of the protein having the amino acid sequence of *Aspergillus ochraceus* oxidoreductase.
- 10 34. A fusion protein comprising the amino acid sequence of *Aspergillus ochraceus* oxidoreductase.
35. An isolated protein wherein the amino acid sequence of said protein is as set forth in SEQ ID NO: 6.
- 15 36. An isolated variant of a protein wherein the amino acid sequence of said protein is as set forth in SEQ ID NO: 6.
37. A purified polypeptide, the amino acid sequence of which comprises SEQ ID NO: 6 with at least one conservative amino acid substitution.
38. A purified polypeptide, the amino acid sequence of which comprises a sequence at least 99% identical to SEQ ID NO: 6.
- 20 39. A purified polypeptide, the amino acid sequence of which comprises a sequence at least 95% identical to SEQ ID NO: 6.
40. A purified polypeptide, the amino acid sequence of which comprises a sequence at least 90% identical to SEQ ID NO: 6.
- 25 41. A purified polypeptide, the amino acid sequence of which comprises a sequence at least 75% identical to SEQ ID NO: 6.
42. A purified polypeptide, the amino acid sequence of which comprises a sequence at least 50% identical to SEQ ID NO: 6.

43. An isolated and purified nucleic acid encoding an enzyme that can catalyze the 11 alpha hydroxylation of 3 keto delta 4,5 steroids (3 keto delta 4 steroids); 3 keto delta 4, 5 delta 6, 7 steroids (3 keto delta 4 delta 6 steroids); 3 keto delta 6, 7 steroids (3 keto delta 6 steroids); or 3 keto delta 1, 2 delta 4, 5 steroids (3 keto delta 1 delta 4 steroids).
44. An isolated and purified nucleic acid of claim 43, wherein said enzyme does not catalyze the 15 beta hydroxylation of 3 keto delta 4,5 steroids; 3 keto delta 4, 5 delta 6, 7 steroids; or 3 keto delta 6, 7 steroids.
45. The isolated and purified nucleic acid of claim 43 or claim 44, wherein said hydroxylation is selected from the group consisting of :
- (a) canrenone to 11 alpha hydroxy canrenone;
 - (b) androstenedione to 11 alpha hydroxy androstenedione;
 - (c) aldona to 11 alpha hydroxy aldona;
 - (d) ADD (1,4 androstenedienedione) to 11 alpha hydroxy ADD;
 - (e) mexrenone to 11 alpha hydroxy mexrenone;
 - (f) 6 beta mexrenone to 11 alpha hydroxy 6 beta mexrenone;
 - (g) 9 alpha mexrenone to 11 alpha hydroxy 9 alpha mexrenone;
 - (h) 12 beta mexrenone to 11 alpha hydroxy 12 beta mexrenone;
 - (i) delta 12 mexrenone to 11 alpha hydroxy delta 12 mexrenone;
 - (j) testosterone to 11 alpha hydroxy testosterone;
 - (k) progesterone to 11 alpha hydroxy progesterone;

- (l) mexrenone 6,7-bis-lactone to 11 alpha hydroxy
mexrenone 6,7-bis-lactone; and
- (m) mexrenone 7,9-bislactone to 11 alpha hydroxy
mexrenone 7,9-bislactone.
- 5 46. The isolated and purified nucleic acid of claim 45,
wherein said hydroxylation is selected from the
group consisting of:
- (a) canrenone to 11 alpha hydroxy canrenone;
- 10 (b) androstenedione to 11 alpha hydroxy
androstenedione;
- (c) aldona to 11 alpha hydroxy aldona; and
- (d) ADD (1,4 androstenedienedione) to 11
alpha hydroxy ADD.
- 15 47. The isolated and purified nucleic acid of
claim 46, wherein said hydroxylation is
from canrenone to 11 alpha hydroxy
canrenone.
- 20 48. A method of expressing a protein that can catalyze the 11 alpha
hydroxylation of 3 keto delta 4,5 steroids; 3 keto delta 4, 5 delta 6, 7
steroids; 3 keto delta 6, 7 steroids; or 3 keto delta 1, 2 delta 4, 5 steroids
comprising;
- (a) transforming or transfecting host cells with an expression cassette
comprising a promoter operably linked to a nucleic acid that
encodes said protein, and
- 25 (b) expressing said protein in said host cells.
49. A method of producing the protein of claim 48, further comprising
the step of recovering said protein.
50. The method of claim 48 or claim 49 wherein said protein is
Aspergillus ochraceus 11 alpha hydroxylase.
- 30 51. The method of claim 50, further comprising expressing an

5 electron donor protein, wherein said electron donor protein
can donate electrons to said protein that can catalyze the 11
alpha hydroxylation of 3 keto delta 4,5 steroids; 3 keto delta
4, 5 delta 6, 7 steroids; 3 keto delta 6, 7 steroids; or 3 keto
delta 1, 2 delta 4, 5 steroids.

52. The method of claim 51 wherein said electron donor
protein is selected from the group consisting of
human oxidoreductase and *Aspergillus ochraceus*
oxidoreductase.

10 53. The method of claim 51 wherein said electron donor
protein is *Aspergillus ochraceus* oxidoreductase.

54. The method of claim 51, wherein the nucleic acid
encoding said steroid 11 alpha hydroxylase and said
electron donor protein are on separate expression
15 cassettes.

55. The method of claim 51, wherein the nucleic acid
encoding said steroid 11 alpha hydroxylase and said
electron donor protein are on the same expression
cassettes.

20 56. The method of claim 54 or claim 55 wherein
said steroid 11 alpha hydroxylase is
Aspergillus ochraceus 11 alpha hydroxylase
and said electron donor protein is human
oxidoreductase.

25 57. The method of claim 54 or claim 55 wherein
said steroid 11 alpha hydroxylase is
Aspergillus ochraceus 11 alpha hydroxylase
and said electron donor protein is
Aspergillus ochraceus oxidoreductase.

30 58. The method of claim 48 wherein said expression cassette is on an
expression vector.

59. The method of claim 58, wherein said expression vector is a
baculovirus.

- 5 60. The method of claim 59, wherein said baculovirus is a nuclear polyhedrosis virus is selected from the group consisting of *Autographa californica* nuclear polyhedrosis virus and *Bombyx mori* nuclear polyhedrosis virus.
61. The method of claim 60, wherein said nuclear polyhedrosis virus is *Autographa californica* nuclear polyhedrosis virus.
62. The method of claim 58 wherein said host cells are insect cells.
- 10 63. The method of claim 62 wherein said insect cells are selected from the group consisting of *Spodoptera frugiperda*, *Trichoplusia ni*, *Autographa californica*, and *Manduca sexta* cells.
- 15 64. The method of claim 63 wherein said insect cells are *Spodoptera frugiperda* cells.
65. The method of any of claims 48 through 64, wherein said *Aspergillus ochraceus* 11 alpha hydroxylase is SEQ ID NO: 2.
66. The method of any of claims 48 through 64, wherein said human oxidoreductase is SEQ ID NO: 4.
- 20 67. The method of any of claims 48 through 64, wherein said *Aspergillus ochraceus* oxidoreductase is SEQ ID NO: 6.
- 25 68. An isolated and purified polypeptide that can catalyze the 11 alpha hydroxylation of 3 keto delta 4,5 steroids (3 keto delta 4 steroids); 3 keto delta 4, 5 delta 6, 7 steroids (3 keto delta 4 delta 6 steroids); 3 keto delta 6, 7 steroids (3 keto delta 6 steroids); or 3 keto delta 1, 2 delta 4, 5 steroids (3 keto delta 1 delta 4 steroids).
- 30 69. An isolated and purified polypeptide claim 68, wherein said enzyme does not catalyze the 15 beta hydroxylation of 3 keto delta 4,5 steroids; 3 keto delta 4, 5 delta 6, 7 steroids; or 3 keto delta 6, 7 steroids.

70. The isolated and purified polypeptide of claim 68 or claim 69, wherein said hydroxylation is selected from the group consisting of :
- 5 (a) canrenone to 11 alpha hydroxy canrenone;
- (b) androstenedione to 11 alpha hydroxy androstenedione;
- (c) aldona to 11 alpha hydroxy aldona;
- (d) ADD (1,4 androstenedienedione) to 11 alpha hydroxy ADD;
- 10 (e) mexrenone to 11 alpha hydroxy mexrenone;
- (f) 6 beta mexrenone to 11 alpha hydroxy 6 beta mexrenone;
- (g) 9 alpha mexrenone to 11 alpha hydroxy 9 alpha mexrenone;
- 15 (h) 12 beta mexrenone to 11 alpha hydroxy 12 beta mexrenone;
- (i) delta 12 mexrenone to 11 alpha hydroxy delta 12 mexrenone;
- (j) testosterone to 11 alpha hydroxy testosterone;
- 20 (k) progesterone to 11 alpha hydroxy progesterone;
- (l) mexrenone 6,7-bis-lactone to 11 alpha hydroxy mexrenone 6,7-bis-lactone; and
- (m) mexrenone 7,9-bislactone to 11 alpha hydroxy mexrenone 7,9-bislactone.
- 25 71. The isolated and purified polypeptide of claim 70, wherein said hydroxylation is selected from the group consisting of:
- (a) canrenone to 11 alpha hydroxy canrenone;

- 5
- (b) androstenedione to 11 alpha hydroxy androstenedione;
- (c) aldona to 11 alpha hydroxy aldona; and
- (d) ADD (1,4 androstenedienedione) to 11 alpha hydroxy ADD.
72. The isolated and purified enzyme of claim 71, wherein said hydroxylation is from canrenone to 11 alpha hydroxy canrenone.
- 10 73. An expression cassette comprising a promoter operably linked to an isolated and purified nucleic acid encoding a polypeptide that can catalyze the 11 alpha hydroxylation of 3 keto delta 4,5 steroids (3 keto delta 4 steroids); 3 keto delta 4, 5 delta 6, 7 steroids (3 keto delta 4 delta 6 steroids); 3 keto delta 6, 7 steroids (3 keto delta 6 steroids); or 3 keto delta
- 15 1, 2 delta 4, 5 steroids (3 keto delta 1 delta 4 steroids).
74. An expression cassette of claim 73, wherein said polypeptide does not catalyze the 15 beta hydroxylation of 3 keto delta 4,5 steroids; 3 keto delta 4, 5 delta 6, 7 steroids; or 3 keto delta 6, 7 steroids.
- 20 75. The expression cassette of claim 73 or claim 74, wherein said hydroxylation is selected from the group consisting of :
- (a) canrenone to 11 alpha hydroxy canrenone;
- (b) androstenedione to 11 alpha hydroxy androstenedione;
- (c) aldona to 11 alpha hydroxy aldona;
- 25 (d) ADD (1,4 androstenedienedione) to 11 alpha hydroxy ADD;
- (e) mexrenone to 11 alpha hydroxy mexrenone;
- (f) 6 beta mexrenone to 11 alpha hydroxy 6 beta mexrenone;

- 5 (g) 9 alpha mexrenone to 11 alpha hydroxy 9 alpha mexrenone;
- (h) 12 beta mexrenone to 11 alpha hydroxy 12 beta mexrenone;
- (i) delta 12 mexrenone to 11 alpha hydroxy delta 12 mexrenone;
- (j) testosterone to 11 alpha hydroxy testosterone;
- (k) progesterone to 11 alpha hydroxy progesterone;
- 10 (l) mexrenone 6,7-bis-lactone to 11 alpha hydroxy mexrenone 6,7-bis-lactone; and
- (m) mexrenone 7,9-bislactone to 11 alpha hydroxy mexrenone 7,9-bislactone.
- 15 76. The expression cassette of claim 75, wherein said hydroxylation is selected from the group consisting of:
- (a) canrenone to 11 alpha hydroxy canrenone;
- (b) androstenedione to 11 alpha hydroxy androstenedione;
- (c) aldona to 11 alpha hydroxy aldona; and
- 20 (d) ADD (1,4 androstenedienedione) to 11 alpha hydroxy ADD.
77. The expression cassette of claim 76, wherein said hydroxylation is from canrenone to 11 alpha hydroxy canrenone.
- 25 78. An expression cassette comprising a promoter operably linked to an isolated and purified nucleic acid encoding *Aspergillus ochraceus* oxidoreductase.
79. An expression cassette of claim 78 wherein said nucleic acid is SEQ ID NO: 06.

80. An expression cassette comprising a heterologous DNA encoding an enzyme from the metabolic pathway for the synthesis of sitosterol to eplerenone wherein said enzyme catalyzes at least one conversion selected from the group consisting of:

- 5 (a) canrenone to 11 alpha hydroxy canrenone;
- (b) androstenedione to 11 alpha hydroxy androstenedione;
- (c) aldona to 11 alpha hydroxy aldona;
- (d) ADD (1,4 androstenedienedione) to 11 alpha hydroxy ADD;
- (e) mexrenone to 11 alpha hydroxy mexrenone;
- 10 (f) 6 beta mexrenone to 11 alpha hydroxy 6 beta mexrenone;
- (g) 9 alpha mexrenone to 11 alpha hydroxy 9 alpha mexrenone;
- (h) 12 beta mexrenone to 11 alpha hydroxy 12 beta mexrenone;
- (i) delta 12 mexrenone to 11 alpha hydroxy delta 12
mexrenone;
- 15 (j) testosterone to 11 alpha hydroxy testosterone;
- (k) progesterone to 11 alpha hydroxy progesterone;
- (l) mexrenone 6,7-bis-lactone to 11 alpha hydroxy mexrenone
6,7-bis-lactone;
- (m) mexrenone 7,9-bislactone to 11 alpha hydroxy mexrenone
20 7,9-bislactone;

and wherein the heterologous DNA is operably linked to control sequences required to express the encoded enzymes in a recombinant host.

25 81. The expression cassette according to claim 80, characterized in that the heterologous DNA coding sequences are selected from the group consisting of the following genus and species: *Aspergillus ochraceus*, *Aspergillus ochraceus*, *Aspergillus niger*, *Aspergillus nidulans*, *Rhizopus oryzae*, *Rhizopus stolonifer*, *Streptomyces*

- 5 *fradiae*, *Bacillus megaterium*, *Pseudomonas cruciviae*,
Trichothecium roseum, *Fusarium oxysporum* *Rhizopus arrhizus*,
Absidia coerula, *Absidia glauca*, *Actinomucor elegans*, *Aspergillus*
flavipes, *Aspergillus fumigatus*, *Beauveria bassiana*,
10 *Botryosphaeria obtusa*, *Calonectria decora*, *Chaetomium cochliodes*,
Corynespora cassiicola, *Cunninghamella blakesleeana*,
Cunninghamella echinulata, *Cunninghamella elegans*, *Curvularia*
clavata, *Curvularia lunata*, *Cylindrocarpon radiculicola*, *Epicoccum*
humicola, *Gongronella butleri*, *Hypomyces chrysospermus*,
15 *Monosporium olivaceum*, *Mortierella isabellina*, *Mucor mucedo*,
Mucor griseocyanus, *Myrothecium verrucaria*, *Nocardia corallina*,
Paecilomyces carneus, *Penicillium patulum*, *Pithomyces*
atroolivaceus, *Pithomyces cynodontis*, *Pycnosporium* sp.,
Saccharopolyspora erythrae, *Sepedonium chrysospermum*,
20 *Stachylidium bicolor*, *Streptomyces hygroscopicus*, *Streptomyces*
purpurascens, *Syncephalastrum racemosum*, *Thamnostylum*
piriforme, *Thielavia terricola*, and *Verticillium theobromae*,
Cephalosporium aphidicola, *Cochliobolus lunatas*, *Tieghemella*
orchidis, *Tieghemella hyalospora*, *Monosporium olivaceum*,
25 *Aspergillus ustus*, *Fusarium graminearum*, *Verticillium glaucum*,
and *Rhizopus nigricans*.

82. The expression cassette according to claim 81, wherein the
genus and species are selected from the group consisting of
25 *Aspergillus ochraceus*, *Aspergillus ochraceus*, *Aspergillus*
niger, *Aspergillus nidulans*, *Rhizopus oryzae*, *Rhizopus*
stolonifer, *Streptomyces fradiae*, *Bacillus megaterium*,
Pseudomonas cruciviae, *Trichothecium roseum*, *Fusarium*
oxysporum *Rhizopus arrhizus*, and *Monosporium*
olivaceum.

- 30 83. The expression cassette according to claim 82,
wherein the genus species is *Aspergillus ochraceus*.

84. A recombinant host cell and progeny thereof comprising at least
one expression cassette according to claim 80.

- 35 85. The recombinant host cell and progeny thereof according to
claim 84, wherein the host is a microorganism.

86. The recombinant host cell and progeny thereof according to claim 85, wherein the host is a bacterium.
- 5 87. A process for making one or more enzymes from the metabolic pathway for the synthesis of sitosterol to eplerenone comprising incubating the recombinant host cell of claim 86 in a nutrient medium under conditions where the one or more enzymes encoded by the heterologous DNA are expressed and accumulate.
- 10 88. A process for the selective oxidation of a compound to an hydroxylated product, which process comprises the steps of: (a) incubating the compound to be hydroxylated in the presence the recombinant host cells of claim 86 under conditions where the compound is hydroxylated and the hydroxylated product accumulates, and (b) recovering the hydroxylated product.
- 15 89. A process for the selective hydroxylation of a compound to an hydroxylated product in vitro, which process comprises the steps of: (a) incubating the compound to be hydroxylated in the presence of the enzymes produced in the process of claim 88 under conditions where the compound is hydroxylated and the hydroxylated product accumulates, and (b) recovering the hydroxylated product.
- 20 90. A host cell harboring an expression cassette of any of claims 73 to 83.
- 25 91. A host cell of claim 90, wherein said expression cassette is integrated into the chromosome of said host cell.
- 30 35

92. A host cell of claim 90, wherein said expression cassette is integrated into an expression vector.
- 5 93. A method of determining the specific activity of a cloned 11 alpha hydroxylase comprising the steps of;
- (a) transforming host cells with an expression vector comprising a nucleic acid that encodes said 11 alpha hydroxylase,
 - (b) expressing said 11 alpha hydroxylase in said host cells;
 - (c) preparing subcellular membrane fractions from said cells,
 - 10 (d) incubating said subcellular membrane fractions microsomes with a steroid substrate, and
 - (e) monitoring conversion of the steroid substrate to its 11 alpha hydroxy steroid counterpart.
- 15 94. The method of claim 93 further comprising transforming host cells with an expression vector nucleic acid that encodes an oxidoreductase, and expressing said oxidoreductase in said host cells.
95. The method of claim 94 wherein said oxidoreductase is human or *Aspergillus ochraceus*.
- 20 96. The method of claim 95 wherein said oxidoreductase is human oxidoreductase.
97. The method of claim 95 wherein said oxidoreductase is *Aspergillus ochraceus* oxidoreductase.
- 25 98. A protein having SEQ ID NO: 2 and variants thereof that are at least 95% identical to SEQ ID NO: 2 and catalyze the 11 alpha hydroxylation of 3 keto delta 4,5 steroids; 3 keto delta 4, 5 delta 6, 7 steroids; 3 keto delta 6, 7 steroids; or 3 keto delta 1, 2 delta 4, 5 steroids, wherein said hydroxylation
- 30 is selected from the group consisting of :

- (a) canrenone to 11 alpha hydroxy canrenone;
- (b) androstenedione to 11 alpha hydroxy androstenedione;
- (c) aldona to 11 alpha hydroxy aldona;
- (d) ADD (1,4 androstenedienedione) to 11 alpha hydroxy ADD;
- 5 (e) mexrenone to 11 alpha hydroxy mexrenone;
- (f) 6 beta mexrenone to 11 alpha hydroxy 6 beta mexrenone;
- (g) 9 alpha mexrenone to 11 alpha hydroxy 9 alpha mexrenone;
- (h) 12 beta mexrenone to 11 alpha hydroxy 12 beta mexrenone;
- (i) delta 12 mexrenone to 11 alpha hydroxy delta 12 mexrenone;
- 10 (j) testosterone to 11 alpha hydroxy testosterone;
- (k) progesterone to 11 alpha hydroxy progesterone;
- (l) mexrenone 6,7-bis-lactone to 11 alpha hydroxy mexrenone 6,7-bis-lactone; and
- (m) mexrenone 7,9-bislactone to 11 alpha hydroxy mexrenone 7,9-bislactone.
- 15
99. A protein of claim 98, which does not catalyze the 15 beta hydroxylation of 3 keto delta 4,5 steroids; 3 keto delta 4, 5 delta 6, 7 steroids; or 3 keto delta 6, 7 steroids.
- 20
100. A purified polypeptide, the amino acid sequence of which is selected from the group consisting of SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25.
101. Purified immunogenic polypeptide, the amino acid sequence of which comprises at least ten consecutive residues of SEQ ID NO: 2.
- 25 102. An isolated and purified antibody having a binding specificity for 11 alpha hydroxylase having an amino acid sequence as shown in SEQ ID NO: 2.

103. The antibody of claim 102 which binds to a protein region selected from the group consisting of
- (a) the N-terminal amino acids 1-10 of SEQ ID NO: 2;
 - (b) the last 10 C-terminal amino acids of SEQ ID NO: 2;
 - 5 (c) amino acids SEQ ID NO: 23;
 - (d) amino acids SEQ ID NO: 24; and
 - (e) amino acids SEQ ID NO: 25.
104. The antibody of claim 102 or claim 103, wherein said antibody is purified on a peptide column, wherein said peptide is selected from the group consisting of:
- (a) the N-terminal amino acids 1-10 of SEQ ID NO: 2;
 - (b) the last 10 C-terminal amino acids of SEQ ID NO: 2;
 - (c) amino acids SEQ ID NO: 23;
 - (d) amino acids SEQ ID NO: 24; and
 - 15 (e) amino acids SEQ ID NO: 25.
105. A purified polypeptide, the amino acid sequence of which is selected from the group consisting of SEQ ID NO: 26.
106. Purified immunogenic polypeptide, the amino acid sequence of which comprises at least ten consecutive residues of SEQ ID NO: 6.
- 20 107. An isolated and purified antibody having a binding specificity for 11 alpha hydroxylase having an amino acid sequence as shown in SEQ ID NO: 6.
108. The antibody of claim 107 which binds to a protein region selected from the group consisting of
- (a) the N-terminal amino acids 1-10 of SEQ ID NO: 6;
 - 25 (b) the last 10 C-terminal amino acids of SEQ ID NO: 6; and
 - (c) amino acids SEQ ID NO: 26.

109. The antibody of claim 107 or claim 108, wherein said antibody is purified on a peptide column, wherein said peptide is selected from the group consisting of:
- 5 (a) the N-terminal amino acids 1-10 of SEQ ID NO: 6;
- (b) the last 10 C-terminal amino acids of SEQ ID NO: 6; and
- (c) amino acids SEQ ID NO: 26.
110. A composition comprising the antibody of claim 102, 103, 104, 107, 108, or 109 and an effective carrier, vehicle, or auxiliary agent.
111. A composition comprising the antibody of claim 102, 103, 104, 107, 108, or 109 and a solution.
- 10 112. The antibody of claim 102, 103, 104, 107, 108, or 109 wherein said antibody is a polyclonal antibody.
113. The antibody of claim 102, 103, 104, 107, 108, or 109 wherein said antibody is a monoclonal antibody.
- 15 114. An antibody of claim 102, 103, 104, 107, 108, or 109 conjugated to an immunoaffinity matrix.
115. A method of using an immunoaffinity matrix of claim 114 to purify a polypeptide from a biological fluid or cell lysate.
116. An antibody of claim 114 wherein said immunoaffinity matrix is SEPHAROSE 4B.
- 20 117. A method of using an immunoaffinity matrix of claim 116 to purify a polypeptide from a biological fluid or cell lysate.
118. A method of using a peptide column to purify an antibody, wherein said peptide is selected from the group consisting of:
- 25 (a) the N-terminal amino acids 1-10 of SEQ ID NO: 2;
- (b) the last 10 C-terminal amino acids of SEQ ID NO: 2;
- (c) amino acids SEQ ID NO: 23;

- (d) amino acids SEQ ID NO: 24; and
- (e) amino acids SEQ ID NO: 25.
- 119 A method of using a peptide column to purify an antibody, wherein said peptide is selected from the group consisting of:
- 5 (a) the N-terminal amino acids 1-10 of SEQ ID NO: 6;
- (b) the last 10 C-terminal amino acids of SEQ ID NO: 6; and
- (c) amino acids SEQ ID NO: 26.
120. A method of detecting a first polypeptide in a biological fluid, wherein said first polypeptide is selected from the group consisting of 11 alpha hydroxylase and oxidoreductase, comprising the
- 10 following steps:
- (a) contacting said fluid with a second polypeptide, having a binding specificity for said first polypeptide, and
- (b) assaying the presence of said second polypeptide to
- 15 determine the level of said first polypeptide.
121. The method of claim 120, wherein said second polypeptide is an antibody.
122. The method of claim 120 or claim 121 wherein said second polypeptide is radiolabeled.
- 20
123. A process for producing an isolated nucleic acid comprising hybridizing SEQ ID NO: 1 to genomic DNA in 6XSSC and 65°C and isolating the nucleic acid detected with SEQ ID NO: 1.
124. The isolated DNA nucleic acid prepared according to the process of
- 25 claim 123.
125. An isolated nucleic acid that specifically hybridizes under highly stringent conditions to the complement of the sequence set forth in SEQ ID NO: 1.

123. A process for producing an isolated nucleic acid comprising hybridizing SEQ ID NO: 5 to genomic DNA in 6XSSC and 65°C and isolating the nucleic acid detected with SEQ ID NO: 5.
- 5 124. The isolated DNA nucleic acid prepared according to the process of claim 123.
125. An isolated nucleic acid that specifically hybridizes under highly stringent conditions to the complement of the sequence set forth in SEQ ID NO: 5.
- 10 126. A DNA construct which alters the expression of a steroid 11 alpha hydroxylase gene not normally expressed in a cell when said DNA construct is inserted into chromosomal DNA of the cell, said DNA construct comprising:
- a) a targeting sequence;
 - b) a regulatory sequence; and
 - c) the structural gene for a steroid 11 alpha hydroxylase.
- 15 127. A host cell harboring the DNA construct of claim 126.
128. Use of a host cell harboring a cloned 11 alpha hydroxylase for the manufacture of a medicament for therapeutic application to treat heart disease, inflammation, arthritis, or cancer.
- 20 129. A composition comprising from about 0.5-500 g/L molasses, 0.5-50 g/L cornsteep liquid, 0.5-50 g/L KH_2PO_4 , 2.5-250 g/L NaCl, 2.5-250 g/L glucose, and 0.04-4 g/L progesterone, pH 3.5-7.
130. A composition comprising from about 10-250 g/L molasses, 1-25 g/L cornsteep liquid, 1-25 g/L KH_2PO_4 , 5-125 g/L NaCl, 5-125 g/L glucose, and 0.08-2 g/L progesterone, pH 4.5-6.5.
- 25 131. A composition comprising from about 25-100 g/L molasses, 2.5-10 g/L cornsteep liquid, 2.5-10 g/L KH_2PO_4 , 12.5-50 g/L NaCl, 12.5-50 g/L glucose, and 0.2-0.8 g/L progesterone, pH 5.5-6.0.
- 30 132. A composition comprising from 50 g/L molasses, 5 g/L cornsteep liquid, 5 g/L KH_2PO_4 , 25 g/L NaCl, 25 g/L glucose, 20 g/L agar, and 0.4 g/L progesterone, pH 5.8.

133. A composition of any of claims 129-132 further comprising from about 4-100 g/L agar.
134. A composition of any of claims 129-132 further comprising from about 10-40 g/L agar.
- 5 135. A composition of any of claims 129-132 further comprising about 20 g/L agar.
- 10 136. Use of the composition of any of claims 129-135 to produce spores from the microorganism selected from the group consisting of *Aspergillus ochraceus*, *Aspergillus niger*, *Aspergillus nidulans*, *Rhizopus oryzae*, *Rhizopus stolonifer*, and *Trichothecium roseum*, *Fusarium oxysporum*, *Rhizopus arrhizus*, *Monosporium olivaceum*, *Penicillium chrysogenum*, and *Absidia coerulea*.
136. Use of the composition of any of claims 129-135 to produce spores from *Aspergillus ochraceus*.

Figures

Figure 1 - Nucleotide and protein sequence of *Aspergillus ochraceus* 11 alpha hydroxylase

5	tggaagtttt tacacttatt atgccggagc cgaaagattc tgagtcgagg gggtggggaa	60
	caacactata agacctacaa ccacttggat ttggtgaatt tacacgggca ttatcaaaac	120
	agccacaagc tgacagctca ttatc atg ccc ttc ttc act ggg ctt ctg cgc	172
	Met Pro Phe Phe Thr Gly Leu Leu Ala	
10		
	att tac cat agt ctc ata ctc gac aac cca gtc caa acc ctg agc acc	220
	Ile Tyr His Ser Leu Ile Leu Asp Asn Pro Val Gln Thr Leu Ser Thr	
15		
	att gtc gta ttg gcg gca gcg tac tgg ctc gca acg ctc cag ccg agc	268
	Ile Val Val Leu Ala Ala Tyr Trp Leu Ala Thr Leu Gln Pro Ser	
20		
	gac ctt cct gag ctg aat ccc gcc aaa cca ttc gag ttc acc aat cgt	316
	Asp Leu Pro Glu Leu Asn Pro Ala Lys Pro Phe Glu Phe Thr Asn Arg	
25		
	cgt cgt gtt cat gag ttt gtt gaa aat agt aag agc ttg ctt gct cgg	364
	Arg Arg Val His Glu Phe Val Glu Asn Ser Lys Ser Leu Leu Ala Arg	
30		
	ggg agg gaa ttg cac ggg cac gag ccg tac aga ctc atg tct gaa tgg	412
	Gly Arg Glu Leu His Gly His Glu Pro Tyr Arg Leu Met Ser Glu Trp	
35		
	gga tcc ttg att gtc ctg ccc cca gag tgc gcc gac gag ctg cgc aac	460
	Gly Ser Leu Ile Val Leu Pro Pro Glu Cys Ala Asp Glu Leu Arg Asn	
40		
	gac cca aga atg gac ttt gag acg ccc acc acc gac gac tcc cac gga	508
	Asp Pro Arg Met Asp Phe Glu Thr Pro Thr Thr Asp Asp Ser His Gly	
45		
	tat atc cct ggc ttc gac gct ctc aac gca gac ccg aac ctg act aaa	556
	Tyr Ile Pro Gly Phe Asp Ala Leu Asn Ala Asp Pro Asn Leu Thr Lys	
50		
	gtg gtc acc aag tac ctc aca aaa gca ttg aac aag ctt act gct ccg	604
	Val Val Thr Lys Tyr Leu Thr Lys Ala Leu Asn Lys Leu Thr Ala Pro	
55		
	atc tcg cat gaa gcg tcc atc gcc atg aaa gcg gtg ctg ggt gac gat	652
	Ile Ser His Glu Ala Ser Ile Ala Met Lys Ala Val Leu Gly Asp Asp	
60		
	cca gat tgg cgt gag atc tac cca gcc aga gac ttg ctc cag ctc gtc	700
	Pro Asp Trp Arg Glu Ile Tyr Pro Ala Arg Asp Leu Leu Gln Leu Val	
65		
	gcc cgg atg tcg aca aga gtg ttc ctt ggc gag gaa atg tgc aat aac	748
	Ala Arg Met Ser Thr Arg Val Phe Leu Gly Glu Glu Met Cys Asn Asn	
70		
	cag gat tgg atc caa acc tca tca caa tac gcg gcc ctt gcc ttc ggt	796
	Gln Asp Trp Ile Gln Thr Ser Ser Gln Tyr Ala Ala Leu Ala Phe Gly	
75		
	gtc ggt gac aag ctt aga ata tac ccg aga atg atc aga ccg ata gta	844
	Val Gly Asp Lys Leu Arg Ile Tyr Pro Arg Met Ile Arg Pro Ile Val	
80		
	cat tgg ttc atg cca tcc tgt tgg gag ctg cgc cga tcg ctg cga cgc	892
	His Trp Phe Met Pro Ser Cys Trp Glu Leu Arg Arg Ser Leu Arg Arg	
85		
	tgc cga cag att ctc acg ccg tac att cac aaa cgc aag tcc ctg aag	940
	Cys Arg Gln Ile Leu Thr Pro Tyr Ile His Lys Arg Lys Ser Leu Lys	
90		
	ggg acc acg gac gag cag ggc aag ccc ctt atg ttt gat gat tcc atc	988
	Gly Thr Thr Asp Glu Gln Gly Lys Pro Leu Met Phe Asp Asp Ser Ile	

5	gag tgg ttc gag cga gag ctg ggt ccc aac cac gac gcg gtc ctg aag Glu Trp Phe Glu Arg Glu Leu Gly Pro Asn His Asp Ala Val Leu Lys 285 290 295	1036
10	cag gtc acg ctc tcc ata gtt gct atc cac acc acg agt gac cta ctc Gln Val Thr Leu Ser Ile Val Ala Ile His Thr Thr Ser Asp Leu Leu 300 305 310	1084
15	ttg cag gcc atg agc gat ctc gcg cag aac ccg aaa gtg cta caa gca Leu Gln Ala Met Ser Asp Leu Ala Gln Asn Pro Lys Val Leu Gln Ala 315 320 325	1132
20	gtg cgc gag gag gtg gtc cga gtg ctg agc acc gag ggg ctc agc aag Val Arg Glu Glu Val Val Arg Val Leu Ser Thr Glu Gly Leu Ser Lys 330 335 340 345	1180
25	gtc tgc ctt cac agt ctc aag ctc atg gac agc gcg ttg aag gaa agc Val Ser Leu His Ser Leu Lys Leu Met Asp Ser Ala Leu Lys Glu Ser 350 355 360	1228
30	cag cgt ctc agg cct acg ctt ctc ggc tcc ttt cgt cgg cag gca acg Gln Arg Leu Arg Pro Thr Leu Leu Gly Ser Phe Arg Arg Gln Ala Thr 365 370 375	1276
35	aat gac atc aag ctg aag agc ggg ttt gtc ata aag aaa ggg act aga Asn Asp Ile Lys Leu Lys Ser Gly Phe Val Ile Lys Lys Gly Thr Arg 380 385 390	1324
40	gtc gtg atc gac agc acc cat atg tgg aat ccc gag tat tac act gac Val Val Ile Asp Ser Thr His Met Trp Asn Pro Glu Tyr Tyr Thr Asp 395 400 405	1372
45	cct ctc cag tac gac ggg tac cgc tac ttc aac aag cgg cag aca ccc Pro Leu Gln Tyr Asp Gly Tyr Arg Tyr Phe Asn Lys Arg Gln Thr Pro 410 415 420 425	1420
50	ggc gag gac aag aac gcg ttg ctc gtc agc aca agc gcc aac cac atg Gly Glu Asp Lys Asn Ala Leu Leu Val Ser Thr Ser Ala Asn His Met 430 435 440	1468
55	gga ttc ggt cac ggc gtt cac gcc tgt cct ggc aga ttc ttc gcc tcc Gly Phe Gly His Gly Val His Ala Cys Pro Gly Arg Phe Phe Ala Ser 445 450 455	1516
60	aac gag atc aag att gcc ttg tgt cat atc atc tta aat tat gag tgg Asn Glu Ile Lys Ile Ala Leu Cys His Ile Ile Leu Asn Tyr Glu Trp 460 465 470	1564
65	cgt ctt cca gac ggc ttc aag ccc cag cct ctc aac atc ggg atg act Arg Leu Pro Asp Gly Phe Lys Pro Gln Pro Leu Asn Ile Gly Met Thr 475 480 485	1612
70	tat ctg gcg gat ccc aat acc agg atg ctg atc agg cca cgc aag gcg Tyr Leu Ala Asp Pro Asn Thr Arg Met Leu Ile Arg Pro Arg Lys Ala 490 495 500 505	1660
75	gag atc gat atg gcg agt tta act gtg tag gtcgaacacg aagtcctgat Glu Ile Asp Met Ala Ser Leu Thr Val *	1710
80	gaagtggttat tggtcagtgg gtgaagcaag tcgcagaaat gtgtaacaat ttataagaat aaaaaa	1770 1776

**Figure 2 - Nucleotide and protein sequence of human
oxidoreductase**

5	atg gga gac tcc cac gtg gac acc agc tcc acc gtg tcc gag gcg gtg Met Gly Asp Ser His Val Asp Thr Ser Thr Val Ser Glu Ala Val	48
10	gcc gaa gaa gta tct ctt ttc agc atg acg gac atg att ctg ttt tcg Ala Glu Glu Val Ser Leu Phe Ser Met Thr Asp Met Ile Leu Phe Ser	96
15	ctc atc gtg ggt ctc cta acc tac tgg ttc ctc ttc aga aag aaa aaa Leu Ile Val Gly Leu Leu Thr Tyr Trp Phe Leu Phe Arg Lys Lys Lys	144
20	gaa gaa gtc ccc gag ttc acc aaa att cag aca ttg acc tcc tct gtc Glu Glu Val Pro Glu Phe Thr Lys Ile Gln Thr Leu Thr Ser Ser Val	192
25	aga gag agc agc ttt gtg gaa aag atg aag aaa acg ggg agg aac atc Arg Glu Ser Ser Phe Val Glu Lys Met Lys Lys Thr Gly Arg Asn Ile	240
30	atc gtg ttc tac ggc tcc cag acg ggg act gca gag gag ttt gcc aac Ile Val Phe Tyr Gly Ser Gln Thr Gly Thr Ala Glu Glu Phe Ala Asn	288
35	cgc ctg tcc aag gac gcc cac cgc tac ggg atg cga ggc atg tca gcg Arg Leu Ser Lys Asp Ala His Arg Tyr Gly Met Arg Gly Met Ser Ala	336
40	gac cct gag gag tat gac ctg gcc gac ctg agc agc ctg cca gag atc Asp Pro Glu Glu Tyr Asp Leu Ala Asp Leu Ser Ser Leu Pro Glu Ile	384
45	gac aac gcc ctg gtg gtt ttc tgc atg gcc acc tac ggt gag gga gac Asp Asn Ala Leu Val Val Phe Cys Met Ala Thr Tyr Gly Glu Gly Asp	432
50	ccc acc gac aat gcc cag gac ttc tac gac tgg ctg cag gag aca gac Pro Thr Asp Asn Ala Gln Asp Phe Tyr Asp Trp Leu Gln Glu Thr Asp	480
55	gtg gat ctc tct ggg gtc aag ttc gcg gtg ttt ggt ctt ggg aac aag Val Asp Leu Ser Gly Val Lys Phe Ala Val Phe Gly Leu Gly Asn Lys	528
60	acc tac gag cac ttc aat gcc atg ggc aag tac gtg gac aag cgg ctg Thr Tyr Glu His Phe Asn Ala Met Gly Lys Tyr Val Asp Lys Arg Leu	576
65	gag cag ctc ggc gcc cag cgc atc ttt gag ctg ggg ttg ggc gac gac Glu Gln Leu Gly Ala Gln Arg Ile Phe Glu Leu Gly Leu Gly Asp Asp	624
70	gat ggg aac ttg gag gag gac ttc atc acc tgg cga gag cag ttc tgg Asp Gly Asn Leu Glu Glu Asp Phe Ile Thr Trp Arg Glu Gln Phe Trp	672
75	ccg gcc gtg tgt gaa cac ttt ggg gtg gaa gcc act ggc gag gag tcc Pro Ala Val Cys Glu His Phe Gly Val Glu Ala Thr Gly Glu Glu Ser	720
80	agc att cgc cag tac gag ctt gtg gtc cac acc gac ata gat gcg gcc Ser Ile Arg Gln Tyr Glu Leu Val Val His Thr Asp Ile Asp Ala Ala	768
	aag gtg tac atg ggg gag atg ggc cgg ctg aag agc tac gag aac cag Lys Val Tyr Met Gly Glu Met Gly Arg Leu Lys Ser Tyr Glu Asn Gln	816
	aag ccc ccc ttt gat gcc aag aat ccg ttc ctg gct gca gtc acc acc Lys Pro Phe Asp Ala Lys Asn Pro Phe Leu Ala Ala Val Thr Thr	864
	aac cgg aag ctg aac cag gga acc gag cgc cac ctc atg cac ctg gaa Asn Arg Lys Leu Asn Gln Gly Thr Glu Arg His Leu Met His Leu Glu	912
	ttg gac atc tcg gac tcc aaa atc agg tat gaa tct ggg gac cac gtg Leu Asp Ile Ser Asp Ser Lys Ile Arg Tyr Glu Ser Gly Asp His Val	960

	305	310	315	320	
5	gct gtg tac cca Ala Val Tyr Pro	gcc aac gac tct Ala Asn Asp Ser	gct ctc gtc aac cag ctg Ala Leu Val Asn Gln Leu	ggc aaa Gly Lys	1008
10	atc ctg ggt gcc Ile Leu Gly Ala	gac ctg gac gtc Asp Leu Asp Val	gtc atg tcc ctg aac aac Met Ser Leu Asn Asn Leu	gat Asp	1056
15	gag gag tcc aac aag aag cac Glu Glu Ser Asn Lys Lys	cca ttc ccg tgc cct acg tcc tac cgc Pro Phe Pro Cys Pro Thr Ser Tyr Arg			1104
20	acg gcc ctc acc tac tac Thr Ala Leu Thr Tyr Tyr	ctg gac atc acc aac Leu Asp Ile Thr Asn	ccg ccg cgt acc aac Pro Pro Arg Thr Asn		1152
25	gtg ctg tac gag ctg gcg cag Val Leu Tyr Glu Leu Ala Gln Tyr	gac tcc ggc gag gcc tcc Ala Ser Ser Ser Gly Glu	gag ccc tcc gag cag gag Pro Ser Glu Gln Glu		1200
30	ctg ctg cgc aag atg gcc tcc tcc Leu Leu Arg Lys Met Ala Ser Ser	gag gcc aag gag ctg tac Gly Glu Gly Lys Glu Leu Tyr			1248
35	ctg agc tgg gtg gtg gag gcc Leu Ser Trp Val Val Glu Ala Arg	cgg agg cac atc ctg gcc atc ctg cag Arg Arg His Ile Leu Ala Ile Leu Gln			1296
40	gac tgc ccg tcc ctg cgg ccc Asp Cys Pro Ser Leu Arg Pro	ccc atc gac cac ctg tgt gag ctg ctg Ile Asp His Leu Cys Glu Leu Leu			1344
45	ccg cgc ctg cag gcc cgc tac Pro Arg Leu Gln Ala Arg Tyr Tyr	tcc atc gcc tca tcc tcc aag gtc Ser Ile Ala Ser Ser Ser Lys Val			1392
50	cac ccc aac tct gtg cac atc His Pro Asn Ser Val His Ile Cys	tgt gcg gtg gtt gtg gag tac gag acc Ala Val Val Val Glu Tyr Glu Thr			1440
55	aag gcc ggc cgc atc aac aag ggc Lys Ala Gly Arg Ile Asn Lys Gly	gtg gcc acc aac tgg ctg cgg gcc Val Ala Thr Asn Trp Leu Arg Ala			1488
60	aag gag cct gcc ggg gag aac ggc Lys Glu Pro Ala Gly Glu Asn Gly	ggt gcg ctg gtg ccc atg ttc Arg Ala Leu Val Pro Met Phe			1536
65	gtg cgc aag tcc cag ttc cgc Val Arg Lys Ser Gln Phe Arg	ctg ccc ttc aag gcc acc acg cct gtc Leu Pro Phe Lys Ala Thr Thr Pro Val			1584
70	atc atg gtg ggc ccc ggc acc ggg Ile Met Val Gly Pro Gly Thr	gtg gca ccc ttc ata ggc ttc atc Val Ala Pro Phe Ile Gly Phe Ile			1632
75	cag gag cgg gcc tgg ctg cga Gln Glu Arg Ala Trp Leu Arg Gln	cag ggc aag gag gtg ggg gag acg Gln Gln Gly Lys Glu Val Gly Glu Thr			1680
80	ctg ctg tac tac ggc tgc cgc Leu Leu Tyr Tyr Gly Cys Arg Arg	tcg gat gag gac tac ctg tac cgg Ser Asp Glu Asp Tyr Leu Tyr Arg			1728
85	gag gag ctg gcg cag ttc cac Glu Glu Leu Ala Gln Phe His	agg gac ggt gcg ctc acc cag ctc aac Arg Asp Gly Ala Leu Thr Gln Leu Asn			1776
90	gtg gcc ttc tcc cgg gag cag Val Ala Phe Ser Arg Glu Gln Ser	tcc cac aag gtc tac gtc cag cac ctg Ser His Lys Val Tyr Val Gln His Leu			1824
95	cta aag caa gac cga gag cac Leu Lys Gln Asp Arg Glu His	ctg tgg aag ttg atc gaa ggc ggt gcc Leu Trp Lys Leu Ile Glu Gly Gly Ala			1872
100	cac atc tac gtc tgt ggg gat His Ile Tyr Val Cys Gly Asp	gca cgg aac atg gcc agg gat gtg cag Ala Arg Asn Met Ala Arg Asp Val Gln			1920
105	aac acc ttc tac gac atc gtg Asn Thr Phe Tyr Asp Ile Val	gct gag ctc ggg gcc atg gag cac gcg Ala Glu Leu Gly Ala Met Glu His Ala			1968

	cag gcg gtg gac tac atc aag aaa ctg atg acc aag ggc cgc tac tcc	2016
	Gln Ala Val Asp Tyr Ile Lys Lys Leu Met Thr Lys Gly Arg Tyr Ser	
	660 665 670	
5	ctg gac gtg tgg agc	2031
	Leu Asp Val Trp Ser	
	675	

Figure 3 - Nucleotide and protein sequence of *Aspergillus ochraceus oxidoreductase*

5	cttatttcgt ttaggaagag caccggttc ggtgtcttc cttaccctct tattcttctt	60
	cttctgactc cctttttgtt attgatcgcc catctcggtg aacatttggg atatctttcc	120
	ctctccccct cccgccccga cctctcttat cttctctcc cgtccagcat ttagctcgcc	180
	atcgaattcg caattctctc ctctgactc ttcatcgctg agcgtctctc tc atg gcg	238
	Met Ala	
10	caa ctc gat act ctc gat ttg gtc gtc ctg gtg gcg ctc ttg gtg ggt	286
	Gln Leu Asp Thr Leu Asp Leu Val Val Leu Val Ala Leu Leu Val Gly	
15	agc gtg gcc tac ttc acc aag ggc acc tac tgg gcc gtc gcc aaa gac	334
	Ser Val Ala Tyr Phe Thr Lys Gly Thr Tyr Trp Ala Val Ala Lys Asp	
20	cct tat gcc tcg gct ggt ccg gcg atg aat gga ggc gcc aag gcc ggc	382
	Pro Tyr Ala Ser Ala Gly Pro Ala Met Asn Gly Gly Ala Lys Ala Gly	
25	aag act cgc gac att gtt cag aaa atg gac gaa act ggc aaa aac tgt	430
	Lys Thr Arg Asp Ile Val Gln Lys Met Asp Glu Thr Gly Lys Asn Cys	
30	gtg att ttc tac ggc tcg caa acc ggt acc gct gag gac tac gcg tcc	478
	Val Ile Phe Tyr Gly Ser Gln Thr Gly Thr Ala Glu Asp Tyr Ala Ser	
35	aga ctg gcc aag gaa ggc tcc cag cga ttc ggt ctc aag acc atg gtg	526
	Arg Leu Ala Lys Glu Gly Ser Gln Arg Phe Gly Leu Lys Thr Met Val	
40	gcc gat ctg gag gac tac gac tac gaa aac ctg gaa aag ttc ccc gag	574
	Ala Asp Leu Glu Asp Tyr Asp Tyr Glu Asn Leu Glu Lys Phe Pro Glu	
45	gac aag gtt gtt ttc ttc gtt ctg gcc act tat ggc gag ggt gaa ccc	622
	Asp Lys Val Val Phe Phe Val Leu Ala Thr Tyr Gly Glu Gly Glu Pro	
50	acg gat aat gcg gtt gaa ttc tac cag ttc gtc acg ggc gaa gat gct	670
	Thr Asp Asn Ala Val Glu Phe Tyr Gln Phe Val Thr Gly Glu Asp Ala	
55	gct ttc gag agc ggc gct acc gcc gac gat aag cct ctg tct tct ctc	718
	Ala Phe Glu Ser Gly Ala Thr Ala Asp Asp Lys Pro Leu Ser Ser Leu	
60	aag tat gtc acg ttt ggt ctg ggt aac aac acc tat gag cac tac aac	766
	Lys Tyr Val Thr Phe Gly Leu Gly Asn Asn Thr Tyr Glu His Tyr Asn	
65	gct atg gtt cgc aat gtg gac gcc gct ctc aca aag ttc ggc gcc caa	814
	Ala Met Val Arg Asn Val Asp Ala Ala Leu Thr Lys Phe Gly Ala Gln	
70	cgc att ggc tct gct ggt gag ggt gac gac ggc gct ggt aca atg gaa	862
	Arg Ile Gly Ser Ala Gly Glu Gly Asp Asp Gly Ala Gly Thr Met Glu	
75	gag gat ttc ctg gcc tgg aag gaa ccc atg tgg gct gcc ctt tct gag	910
	Glu Asp Phe Leu Ala Trp Lys Glu Pro Met Trp Ala Ala Leu Ser Glu	
80	gcg atg aac ctg caa gag cgc gat gcg gtc tac gag ccg gtc ttc aat	958
	Ala Met Asn Leu Gln Glu Arg Asp Ala Val Tyr Glu Pro Val Phe Asn	
85	gtc acc gag gac gag tcc ctg agc ccc gaa gat gag aac gtt tac ctc	1006
	Val Thr Glu Asp Glu Ser Leu Ser Pro Glu Asp Glu Asn Val Tyr Leu	
90	ggg gag ccc act caa ggt cat ctc caa ggc gag ccc aag ggc ccg tac	1054
	Gly Glu Pro Thr Gln Gly His Leu Gln Gly Glu Pro Lys Gly Pro Tyr	
95	tct gcg cac aac ccg ttc atc gct ccc atc tcc gaa tct cgt gaa ctg	1102
	Ser Ala His Asn Pro Phe Ile Ala Pro Ile Ser Glu Ser Arg Glu Leu	

5	ttc aac gtc aag gac cgc aac tgt ctg cac atg gaa atc agc atc gcc Phe Asn Val Lys Asp Arg Asn Cys Leu His Met Glu Ile Ser Ile Ala 295 300 305	1150
	ggg agc aac ctc act tac cag act ggt gac cac atc gct gtt tgg ccc Gly Ser Asn Leu Thr Tyr Gln Thr Gly Asp His Ile Ala Val Trp Pro 310 315 320	1198
10	acc aac gcc ggt tcc gag gtc gat cgg ttc ctg cag gct ttt ggt ctc Thr Asn Ala Gly Ser Glu Val Asp Arg Phe Leu Gln Ala Phe Gly Leu 325 330 335	1246
15	gaa gga aag cgc cac tcc gtc atc aac att aag ggt atc gat gtg acc Glu Gly Lys Arg His Ser Val Ile Asn Ile Lys Gly Ile Asp Val Thr 340 345 350	1294
20	gct aag gtt ccg att ccc act cct acg acc tat gac gcc gca gtt cgc Ala Lys Val Pro Ile Pro Thr Thr Tyr Asp Ala Ala Val Arg 355 360 365	1342
25	tac tac ctg gaa gtc tgt gcc ccc gtt tcc cgt cag ttt gtc tcg act Tyr Tyr Leu Glu Val Cys Ala Pro Val Ser Arg Gln Phe Val Ser Thr 375 380 385	1390
30	ctc gct gcc ttt gcc cct gat gaa gcg acc aag gcg gag atc gtt cgt Leu Ala Ala Phe Ala Pro Asp Glu Ala Thr Lys Ala Glu Ile Val Arg 390 395 400	1438
35	ttg ggt ggc gac aag gac tat ttc cat gag aag att acc aac cga tgc Leu Gly Lys Asp Lys Asp Tyr Phe His Glu Lys Ile Thr Asn Arg Cys 405 410 415	1486
40	ttc aac atc gct cag gct ctc cag agc atc acg tcc aag cct ttc acc Phe Asn Ile Ala Gln Ala Leu Gln Ser Ile Thr Ser Lys Pro Phe Thr 420 425 430	1534
45	gcc gtc ccg ttc tcc ctg ctt atc gaa ggt atc acc aag ctt cag ccc Ala Val Pro Phe Ser Leu Leu Ile Glu Gly Ile Thr Lys Leu Gln Pro 435 440 445 450	1582
50	cgt tac tac tcg atc tcc tcg tct tcc ctg gtt cag aag gac aag att Arg Tyr Tyr Ser Ile Ser Ser Ser Ser Leu Val Gln Lys Asp Lys Ile 455 460 465	1630
55	agc att acc gcc gtt gtg gag tcg gtt cgc ttg cct ggt gag gaa cac Ser Ile Thr Ala Val Val Glu Ser Val Arg Leu Pro Gly Glu Glu His 470 475 480	1678
60	att gtc aag ggt gtg acc acg aac tat ctt ctc gcg ctc aag gaa aag Ile Val Lys Gly Val Thr Thr Asn Tyr Leu Leu Ala Leu Lys Glu Lys 485 490 495	1726
65	caa aac ggc gag cct tcc cct gac ccg cac ggc ttg act tac tct atc Gln Asn Gly Glu Pro Ser Pro Asp Pro His Gly Leu Thr Tyr Ser Ile 500 505 510	1774
70	act gga ccc cgt aac aag tac gat ggc atc cat gtc ccc gtt cac gtc Thr Gly Pro Arg Asn Lys Tyr Asp Gly Ile His Val Pro Val His Val 515 520 525 530	1822
75	cgc cac tcg aac ttc aaa ttg ccc tcg gat ccc tcg cga cct gtg atc Arg His Ser Asn Phe Lys Leu Pro Ser Asp Pro Ser Arg Pro Val Ile 535 540 545	1870
80	atg gtt gga ccc ggt act ggt gtt gct cct ttc cgt ggg ttt atc cag Met Val Gly Pro Gly Thr Gly Val Ala Pro Phe Arg Gly Phe Ile Gln 550 555 560	1918
85	gag cgt gct gcc ttg gcc gcg aag ggc gag aag gtc gga act acc ttg Glu Arg Ala Ala Leu Ala Ala Lys Gly Glu Lys Val Gly Thr Thr Leu 565 570 575	1966
90	ctt ttc ttc ggc tgc cgt aag tcc gac gaa gat ttc ttg tac aag gat Leu Phe Phe Gly Cys Arg Lys Ser Asp Glu Asp Phe Leu Tyr Lys Asp 580 585 590	2014
95	gaa tgg aag act ttt cag gag cag ctt ggc gac tcg ctc aag atc atc Glu Trp Lys Thr Phe Gln Glu Gln Leu Gly Asp Ser Leu Lys Ile Ile 595 600 605 610	2062
100	act gcc ttc tct cgt gaa tcg gct gag aaa gtc tac gtc cag cac agg Thr Ala Phe Ser Arg Glu Ser Ala Glu Lys Val Tyr Val Gln His Arg 615 620 625	2110
105	ctg cgt gag cat gcc gag ctg gtc agt gac ctg ctg aag cag aaa gcc	2158

	Leu Arg Glu His Ala Glu Leu Val Ser Asp Leu Leu Lys Gln Lys Ala	
	630 635 640	
5	act ttc tat gtt tgc ggt gac gct gcc aac atg gcc cgt gaa gtc aac Thr Phe Tyr Val Cys Gly Asp Ala Ala Asn Met Ala Arg Glu Val Asn 645 650 655	2206
10	ctc gtg ctt ggg caa atc att gcc aag cag cgc ggt ctc cct gcc gag Leu Val Leu Gly Gln Ile Ile Ala Lys Gln Arg Gly Leu Pro Ala Glu 660 665 670	2254
15	aag ggc gag gag atg gtg aag cac atg cgc agc agc ggc agc tac cag Lys Gly Glu Glu Met Val Lys His Met Arg Ser Ser Gly Ser Tyr Gln 675 680 685 690	2302
	gac gat gtc tgg tcc taa aa Asp Asp Val Trp Ser *	2322
	695	

Figure 4 - Amino acid homology alignment of *A. ochraceus* 11 alpha hydroxylase with the top 10 BLAST hits from GenBank

5	CAA75565	1	MANHSSSYHFEYKDHSTVL	1	LMSEKPVILPSLILGTCAVLLCIQWLK--PQPLIM
	CAB91316	1	-----MERLDIKSI	1	DDPSATPFYSLVTAFLAVVVSLQGRPF-PKNIKH
	CAB56503	1	-----	1	LLPCFILSKTTKKGQNSQYS-NHDELP
	AAB94588	1	-----MVMEHNNHTPF	1	SIYFITSILFIFFVFFKLQVRS--DSKTS-STCKLP
	pMON45624	1	-----MPFFTGLLAIVHSL	1	LIDNFEVQTLSTIVLAAAY--LATLQ--PSDLPE
10	CAA75566	1	-----MSIFNMITSYAGS	1	QLLPFYIAIFVFTLVPAIRFSWLELRK-GSVVPL
	AAD34552	1	-----MTVDALTQPHLLSL	1	AWNDTQQHGSWFAPLVTTSSAGLLCLLLLCSSGR--RSDLPV
	CAA75567	1	-----	1	-----
	CAA76703	1	MSKSNSMNSTSHETLFQQL	1	VLGLDRMPLMDVHWLIYVAFGAWLCSYVIHVLSSSSTVKVP
	CAA57874	1	-----	1	-----
15	CAA91268	1	-----MALLIL	1	SLVISIFTFFIYIILARRERKLRKIGLSGPEPH
	CAA75565	56	VNKRKFGELSNV	56	ANRDETFGARQLLEKSLKMSDPKPFPRINGVGE
	CAB91316	45	LNKKGPLEFSDTP	45	PKKEFVYGSRLANWFKANFNKPCRVHSDFGAIVLPFRMNGTIN
	CAB56503	28	PGIPQIPILGNAP	28	QOLSGGH-THETURDLAKKYGLMHLK-LGEVSTIVASSQIEETFR
	AAB94588	45	PGERTLPLIGNIT	45	QIVGSLPVHYDKNLADKYGLMHLK-LGEVSNITVTSIEMQFIMK
20	pMON45624	46	LNKAKPFETNRR	46	RVHEFVENSRLDARGRELHGHEFYRLMSWGSLLVLP
	CAA75566	48	ANFDP-SLFGTG	48	ETRRSVKLSREITAKRSLFPNEPFLITWGEVLLLP
	AAD34552	56	FNEKTWWELTT	56	MAKRLSDANAPSWIESWFSQN-DKPIRFFIVESGYCTILHSSMDPFRK
	CAA75567	1	-----	1	-----
	CAA76703	61	VVGYSVFEPTWLL	61	LRVWEGGSLLGOSYNKFKDSIFQVRKLGTD
25	CAA57874	1	-----	1	-----
	CAA91268	43	WFLGNLKQTAER	43	RENLGDDANRFNLFELHEQYGETFGIY-YGSQMN
	CAA75565	116	NEKUS	116	ETMAA--FKWFYAHLPGEG--FREGTNESTIMKLARHQT--
	CAB91316	105	DDRUS	105	TRWT--YKAFEGHLPDEEG--FGEASRESHTVQIMRDT--
	CAB56503	86	THDIL	86	ADRPSNLESPNIVSYDSMDVVSYPGNYWQRLRMISME
30	AAB94588	104	THDIL	104	ADRPSNLESPNIVSYDSMDVVSYPGNYWQRLRMISME
	pMON45624	106	DPRDE	106	ETPT--TDDSHGYIPEDA-----LN-ADPNITKVTKY
	CAA75566	107	DPRDE	107	SKAA--MQDNHAGIPDET--VALVGREDQITQARKQL--
	AAD34552	115	MKE	115	CMYKFLG--TDFHSHLPEDG--FKEVTRDA
	CAA75567	1	-----	1	-----
35	CAA76703	121	LS---	121	QDKTRSVEPFINDFAEQYT--RGMVFLQSDLOM
	CAA57874	1	-----	1	-----
	CAA91268	102	KNFSN	102	SDRS--VPSIYEANQLTASLLMNSYSGWRHTRSAIAPIS-TGKKAQETIN
	CAA75565	170	ECALV	170	UKDLYTDSP--EHDITAKDANMLMARITSEVFL
	CAB91316	159	QETSMAMEANL	159	PKAANGESTINDRSKILPIVARISSFVFLGEELCR
45	CAB56503	146	EEVLN	146	FIKSTG---SKEG-TRINDSKEISLLINGITFAAF
	AAB94588	163	EEVAEL	163	VKKIATAASEEGGSIFNLQSIYSSTFG-AAFAAF
	pMON45624	156	HEASIA	156	KAVLGDDP--DRETYPARILLQVAFMSTFVFL
	CAA75566	161	RESTL	161	AVSNLFGETT--EIRAIRKPAILEDIARISSEFYL
	AAD34552	169	NEASGI	169	HTTFGDSN--EHTVPVYNOCLEVTETVHEFM
50	CAA75567	17	MKTSFR	17	WRPRTS-----KSSVSLYDMMLRTVALLSG
	CAA76703	173	ELDYAL	173	TKEMPDMKNDENVDDISSIMRLISFSAFVFL
	CAA57874	1	-----	1	-----
	CAA91268	159	SKVDLF	159	EDIREKAS--SGQKWDHYDDFQGLTLDVIG
	CAA75565	227	VIAFRAVE	227	RLRLWE-SWLRVVQWFMEHCTQSALVQEA
55	CAB91316	218	HDGFGAAE	218	RLRLWE-AALREIVHWFDESCQRAADVERVS
	CAB56503	202	AVAEPNIAD	202	WFPSL-KFLQLISTSKYKIEIKHQFDVIVET
	AAB94588	223	ILGGFS	223	VADLYPSS-RVFQMMGATG-KLEKVHRTVTVLQ
	pMON45624	213	ALAFG	213	VGDKLRIYE-RMLRPIVHWFENESWELFRS
	CAA75566	218	TNFYTAST	218	LEMLE-RSIRPLAHWFLEECRKPOERTDAICIT
60	AAD34552	226	VTMAI	226	QARCLRLWE-VILRELVHWLEBQAKLE
	CAA75567	70	VQCVS	70	IIRDCLFMS-PVLREITGFDFSVRSVRRH
	CAA76703	232	ESLFI	232	TGFLRVVE-HILRFFIAPLLESYRTL
	CAA57874	1	-----	1	-----
	CAA91268	217	UKITIN	217	NFTYFHSSSPGTPHLESTLQIHTTGC
65	CAA75565	227	VIAFRAVE	227	RLRLWE-SWLRVVQWFMEHCTQSALVQEA
	CAB91316	218	HDGFGAAE	218	RLRLWE-AALREIVHWFDESCQRAADVERVS
	CAB56503	202	AVAEPNIAD	202	WFPSL-KFLQLISTSKYKIEIKHQFDVIVET
	AAB94588	223	ILGGFS	223	VADLYPSS-RVFQMMGATG-KLEKVHRTVTVLQ
	pMON45624	213	ALAFG	213	VGDKLRIYE-RMLRPIVHWFENESWELFRS

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CAA75565 283 ERTGEKV-TYNANBELDDLAREK--GVGYCPACACTSLSVAA--LHSTIDFFTFQVFCI
 CAB91316 274 ---GGKA-EHDCAIPFFERTA--K--CKYYLPAAVAVLSVA--LHSTIDLTQCVQNL
 CAB56503 259 G--EKKE-DLVVLLNIQRRNDFE--AFLGCKNHKAIIFNFPSAGTETSSTVMDWACEN
 AAB94588 276 R--EAVE-DLVVLLKFKQES--E--FRLTCDNKAQIQTIFIGGGETSSSVMEWSSEI
 pMON45624 269 DEQEKPL-MFDGSIKFEREL----SPNHCAVLKVTLSIVA--LHSTIDLTQCVQNL
 CAA75566 274 IAAQPLPVFHCAIDSEQEAAGTASAFCPVIFITLSILA--LHSTIDLTQCVQNL
 AAD34552 282 RAKSIEPPRYVLSHONFEDTAK---SKWYCAAGATLMDFA--LYGTDDLIGSIVCI
 CAA75567 129 TLLAQTEGRTFTISLLRLHP-EELRTPEQVGLVNLVSEMA--LHSTIDLTQCVQNL
 CAA76703 282 ---SDGN---EILSMDRAATGEE-KQIDNIACRMILDSIAS--LHSTIDLTQCVQNL
 CAA57874 44 --LEDPTMLDHLNNGRNEHIA---DDVELQLLHMTIATV--TVTFSSSTQAHYDI
 CAA91268 277 RRRGEGSDSVLLKLLLNREDDKS-KPMTKQEVINCPFFLLAGYETTTATCTYCSYL

CAA75565 338 AONPELIEPLREEIIAVLGKQ-----KSPNSLYNLFMLVLRSSFLK-----
 CAB91316 324 MONPEEIAPLREEMIQVLESEG-----KRTTSIYNNFLDSVIRSSFWK-----
 CAB56503 314 IKNFIVYKKAQEEARKFNBEEN-----VDETKHOLPYLOAVIKETFLH-----
 AAB94588 329 IRNRVMEYEAQAEVRRVYDSKY-----VDETEHQLIYKTHIKETMLH-----
 pMON45624 321 AONKVLCAVREYVRVLTSE-----LSFVGHSLFLMECALYSSALP-----
 CAA75566 332 GRHPYIEPLFQEVQLDREE-----KRTTILFKMPLLESALVESQMK-----
 AAD34552 336 VRHFHLEPLFDEIRTVGGQ-----ATPASLYKFLDLDQLESFVVK-----
 CAA75567 186 VRHPYIEPLFDEKQTFGPDVSPDICINBALSRHKLIFIRVIFWC-----
 CAA76703 333 CACPFYIEPLFDEKSYGAS-----MDTANRFHKLIFELLESIFN-----
 CAA57874 96 MAHPYITILEEESPRDPNGN-----ETDSTVANDKILFELLESIFN-----
 CAA91268 336 SEYENVOAKMEFIMEAKENG-----LTYDSIHMVYLCVYHITLIFY-----

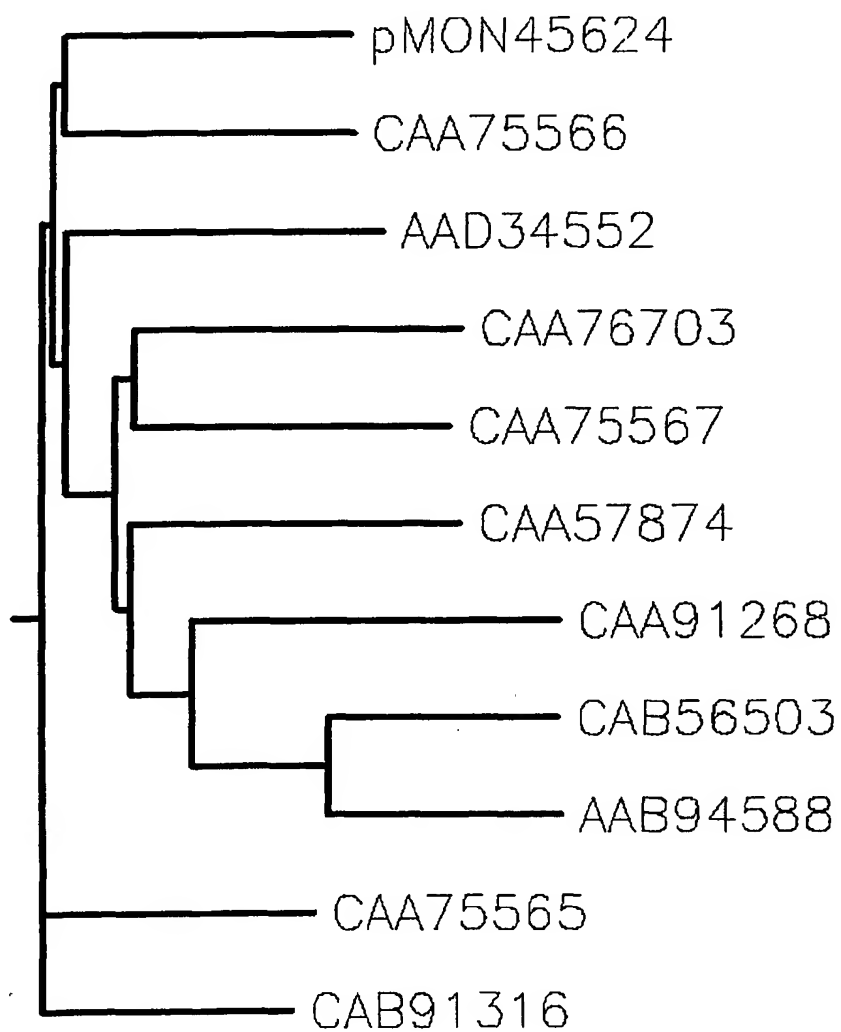
CAA75565 384 -----IAIASMFFTHHVKLS--VILFNNKLTLSAHOHW-----DEEYKQ
 CAB91316 370 -----TGVASRFFYAKEYVLS--TFHPEGGFVASSDHW-----NSGVVEQ
 CAB56503 361 -----PVPLLLFECRECKIK--YTHISKSRVILNWAIGR-----DNYAIE
 AAB94588 376 -----PVPLLLFVSRRCCIN--EYELSKTRIILNWAIGR-----NKKYGE
 pMON45624 367 -----TLGSRFQATMILKLS--FVKEFTFVVLDSHMM-----NRYATL
 CAA75566 378 -----GSIVNFFVVTETLSS--LTLEKATFLNNDNRD-----DKINDN
 AAD34552 382 -----VECATNFYALQCVIFSN--TFLEKELVAAADRS-----NRYVSP
 CAA75567 238 -----STFVTPSRVMKSNLSEN--IKLCRTSTAFF--EAIHMSSETPTFSFSSS
 CAA76703 379 -----VFLLENLYHQSNTLSD--TNITSTRIA--PSHAPLQ-----DSAHVPC
 CAA57874 152 KNYKLCESLTGHSNPRTIATKKIPDCTFVEKTNLEPNTCSHK-----DHKLLEN
 CAA91268 382 -----PHFSFIEFLCRELTIR--QFYPPFAIVVCLPETHR-----NRYVND

CAA75565 427 ELK-----FDGYRFNMREP--GIESKAQLVSAITPHNGFYLHA--PGRFF--SEEIFI
 CAB91316 413 AEK-----ADRPFRLRMETPGACHENVAQLVSAITPHNGFYLHA--PGRFF--SEEIFI
 CAB56503 404 PEK-----FNPDPFLES-----S--VDFKGNSTFYLPFGGRPIPTITFALANTEL
 AAB94588 419 TES-----FKPEFPLNS-----S--IDFRGDFEFIEFCAARRICFCITFIPNIEL
 pMON45624 410 ELQ-----YDGYFVFNKQTP--REDKNALLVSTANHMGGFCHVHACPGPFF--SNEIFI
 CAA75566 421 PEV-----YMPYRFYDMRSEA--GHDHGAQLVSTANHMGGFCHVHACPGPFF--SNEIFI
 AAD34552 425 EAK-----YDGYFVFNKQTP--REDKNALLVSTANHMGGFCHVHACPGPFF--SNEIFI
 CAA75567 289 BENPSRPFDCFFVNLNLSIK--DGSQHQAAICGFDYLIENHCHACRORFF--ISEIYN
 CAA76703 423 PTP-----PTFDPGFYSKHS--NYAQKYLFSM--DSSNMAFQYKYACPGPFF--SNEIFI
 CAA57874 205 PFQ-----FDGLFPHKMKAP--EHEKRYMYSSSGTDLSSAFRRACPGPFF--SNEIFI
 CAA91268 425 PEE-----PHPEPFENWE-----E-----KSSSLKWIPFTVPRYVEMREMEFET

CAA75565 480 AUSHILLKYLFHPV-----EGSSMEPRKYENNNANNTARI--SRRER--EETAI-----
 CAB91316 468 AUVHLLLYNE--RIP-----EGSDPKIRTF--FSAGVDLSLVEYNQEQ--PEHEL-----
 CAB56503 449 FLAQLLFHFD--QS-----NTEKLNKKE--SRVIVRREDDLYTPVNFS--SSSPA-----
 AAB94588 464 FLAQLLYHFDHKL--PNMKMNEELDMTE--SNITLRRONDLC--IPIT-----LP-----
 pMON45624 463 ALCHILLYNE--RIP-----DGFKPQPLNIG--TYLADENTEMLIRPK--AEDMASLTV
 CAA75566 474 ALCHILLYNE--RIP-----PDTETKPDTR--TIKSS--VTDILIRFESVELDLEAI--
 AAD34552 478 MLAYLLIRYDMRV-----PDEPLQYRHSFSL--IHTLTMRRFD--EDRLPGSL
 CAA75567 347 ILIELLAKYIFRL--E-----DGKPGPELMRV--TETRLDTKAG--EMRRF-----
 CAA76703 479 TLAILLLQFERRP-----DGKGRPRNITIDSDYIPDRARLQYRKS--LRDE-----
 CAA57874 258 IYABELLYNIIELP-----DGLSRPKNIEFVSLASLNACANA-----
 CAA91268 468 TIVKLLDTFEL--OF-----EGEADLIPDCN--VIMRPNDPVR--HURFN-----

CAA75565 (SEQ ID NO: 27)
 CAB91316 (SEQ ID NO: 28)
 CAB56503 (SEQ ID NO: 29)
 AAB94588 (SEQ ID NO: 30)
 pMON45624 (SEQ ID NO: 02)
 CAA75566 (SEQ ID NO: 31)
 AAD34552 (SEQ ID NO: 32)
 CAA75567 (SEQ ID NO: 33)
 CAA76703 (SEQ ID NO: 34)
 CAA57874 (SEQ ID NO: 35)
 CAA91268 (SEQ ID NO: 36)

**Figure 5 - Phylogenetic tree showing the relatedness of
Aspergillus ochraceus 11 alpha hydroxylase to the top 10
BLAST hits from GenBank**



**Figure 6 – Percent homology of *Aspergillus ochraceus* 11
alpha hydroxylase to the top 10 BLAST hits from GenBank**

Accession Number	Species	% ID to 11a OH
CAB91316	<i>Neurospora crassa</i>	40
CAA76565	<i>Gibberella fujikuroi</i>	37
CAA75566	<i>Gibberella fujikuroi</i>	37
AAD34552	<i>Aspergillus terreus</i>	29
CAA75567	<i>Gibberella fujikuroi</i>	24
CAA57874	<i>Fusarium oxysporum</i>	24
CAA76703	<i>Gibberella fujikuroi</i>	23
CAB56503	<i>Catharanthus roseus</i>	14
AAB94588	<i>Glycine max</i>	14
CAA91268	<i>Caenorhabditis elegans</i>	12

Figure 7 – Amino acid homology alignment of *A. ochraceus* and human oxidoreductase to NADPH cytochrome P450 reductases from *A. niger*, mouse, and *S. cerevisiae*

5	PMON45605	1	MGDSHVDTSTSEAEEVSLFMTINILFSLIVLLTTHFLFRFEEVFETRAVLL
	human	1	MGDSHVDTSTSEAEEVSLFMTINILFSLIVLLTTHFLFRFEEVFETRAVLL
	mouse	1	MGDSHEDTSATVPPAAEEVSLFSTTTIVLFSLIVGVLTWFFIFKFFFIPIFESHIQTT
	PMON45632	1	--MAQLDTLLCLLALLVGSVAYFKG-----FYAAAFDPA--SAGPAWNGG
	niger	1	--MAQLDTLLCLLALLVGSVAYFKG-----FYAAAFDPA--SAGPAWNGG
10	yeast	1	--MPFGILNLTFTLLGLVLAVLLYMKRN-----STKEILMSDDG-----DI--
	PMON45605	61	TSSVRESFVEEHKPTGRNIIIFYGSGTGTAEFAHLSFD--AHFYMFETASAEVW
	human	61	TSSVRESFVEEHKPTGRNIIIFYGSGTGTAEFAHLSFD--AHFYMFETASAEVW
	mouse	61	PPVRESFVEEHKPTGRNIIIFYGSGTGTAEFAHLSFD--AHFYMFETASAEVW
	PMON45632	47	AKAGTRDITQKDETKICVIFYGSGTGTAEFYSLAFEGSOFFLITVALLSDYV
15	niger	47	AKAGTRDITQKDETKICVIFYGSGTGTAEFYSLAFEGSOFFLITVALLSDYV
	yeast	42	VEISGNRDIAVVTENNKVLLIYASPTGTAEFYKPSHELVAENUNVCAVNVNPF
	PMON45605	120	ADLSSLPRIENALTFCHATYGEEDFTDWAOCFYLLWL-----ST-----ETELAVVF
	human	120	ADLSSLPRIENALTFCHATYGEEDFTDWAOCFYLLWL-----ST-----ETELAVVF
	mouse	120	ADLSSLPRIENALTFCHATYGEEDFTDWAOCFYLLWL-----ST-----ETELAVVF
20	PMON45632	107	ENLEKFLPCHIVFVLLATYGEEDFTDWAOCFYLLWL-----ST-----ETELAVVF
	niger	107	ENLEKFLPCHIVFVLLATYGEEDFTDWAOCFYLLWL-----ST-----ETELAVVF
	yeast	102	ESLNDVIV-----ITSIFLSTFGEGLFPFGVNHLEFICN-----AGAG-----ALNLRV
25	PMON45605	169	AVFGLGNKTYEHFNAGTYVDLEELJACRIFFLLJEL--D--N--EEFFITRETFPAV
	human	169	AVFGLGNKTYEHFNAGTYVDLEELJACRIFFLLJEL--D--N--EEFFITRETFPAV
	mouse	169	AVFGLGNKTYEHFNAGTYVDLEELJACRIFFLLJEL--D--N--EEFFITRETFPAV
	PMON45632	165	VTFGLGNKTYEHFNAGTYVDLEELJACRIFFLLJEL--D--N--EEFFITRETFPAV
	niger	164	VTFGLGNKTYEHFNAGTYVDLEELJACRIFFLLJEL--D--N--EEFFITRETFPAV
30	yeast	148	NMFGLGSIYSEFFITCAAKAEHHSAAASIPFGKLEAEEAATTDETNAKLSILEVL
	PMON45605	228	CEHFGVEATGEESSTROYELVHT-----IDAAKVNIMEMPLPS-----ETTP
	human	228	CEHFGVEATGEESSTROYELVHT-----IDAAKVNIMEMPLPS-----ETTP
	mouse	228	CEHFGVEATGEESSTROYELVHT-----IDAAKVNIMEMPLPS-----ETTP
	PMON45632	225	SEAMNLC---ERDANYSVPVFNTEESLSPEDENV/LGSEPTQCHLO-----GEPFG
35	niger	224	SEAMNLC---ERDANYSVPVFNTEESLSPEDENV/LGSEPTQCHLO-----GEPFG
	yeast	208	KDELHLR---EQEAKFTSQFYTVLNL---EITDSVSLGSPSAHYLPShQLNRNADGILG
40	PMON45605	275	PFDANKPFLAAVTNFKLNQTEFHLNHELDISDKIRYESJHVA/YPAHDSALVHL
	human	275	PFDANKPFLAAVTNFKLNQTEFHLNHELDISDKIRYESJHVA/YPAHDSALVHL
	mouse	275	PFDANKPFLAAVTNFKLNQTEFHLNHELDISDKIRYESJHVA/YPAHDSALVHL
	PMON45632	273	PYSAHNPFLAETSESSEFNNVDFNCLHMETSIAGSNLSYQTDGDLA/WFTAGSETR
	niger	272	PYSAHNPFLAETSESSEFNNVDFNCLHMETSIAGSNLSYQTDGDLA/WFTAGSETR
45	yeast	262	PFDLSQFYIAPIVKSSELPSSNDNCITSEFELSGENIKYSTGDLA/WFTAGSETR
	PMON45605	335	GKILCAI---LDVVMISLNLDEESNKHHPFFCPTSYFTALTYVLDITNPRTNVLYELACY
	human	335	GKILCAI---LDVVMISLNLDEESNKHHPFFCPTSYFTALTYVLDITNPRTNVLYELACY
	mouse	335	GKILCAI---LDVVMISLNLDEESNKHHPFFCPTSYFTALTYVLDITNPRTNVLYELACY
	PMON45632	333	LQAFLEGRKRSVINKGIC---VTAFVETFTPTTDAAVRYYLEVCAVSRQFVATLAF
50	niger	332	LQAFLEGRKRSVINKGIC---VTAFVETFTPTTDAAVRYYLEVCAVSRQFVATLAF
	yeast	322	LSIFNL---PETIFDLKFLC---PTVVPFETPTTIGAKKHVLEIGVSRQFSSILFF
55	PMON45605	393	AS-EPSEQELLRKMASSSGEGKELYLS/VVEARRHILAILQCE-SLFPIDHLCCELLPF
	human	393	AS-EPSEQELLRKMASSSGEGKELYLS/VVEARRHILAILQCE-SLFPIDHLCCELLPF
	mouse	393	AS-EPSEQELLRKMASSSGEGKELYLS/VVEARRHILAILQCE-SLFPIDHLCCELLPF
	PMON45632	391	APDEANKAEIVPLGDKDYFHEKITNRCFNISQ---ALQSITSKF-FTAVFSLLEGITV
	niger	390	APMRKRRLCVWVAG-LFPFGHOPMLOHSD---ALQSITSKF-FSAVEFSLLEGITV
60	yeast	378	APNADVKNLTLLSKDKQFVELTSKFNINID---ALKYLSGAKWDNVNQFVLSVQ
	PMON45605	451	LCARYYSIASSSKVHPNSVHTCAVVVEYETK---AGRINKGVATNMLRAKEH---AGE--
	human	451	LCARYYSIASSSKVHPNSVHTCAVVVEYETK---AGRINKGVATNMLRAKEH---AGE--
	mouse	451	LCARYYSIASSSKVHPNSVHTCAVVVEYETK---AGRINKGVATNMLRAKEH---AGE--
	PMON45632	448	LCFRYYSIASSSKVHPNSVHTCAVVVEYETK---AGRINKGVATNMLRAKEH---AGE--
65	niger	446	LCFRYYSIASSSKVHPNSVHTCAVVVEYETK---AGRINKGVATNMLRAKEH---AGE--
	yeast	436	LCFRYYSIASSSKVHPNSVHTCAVVVEYETK---AGRINKGVATNMLRAKEH---AGE--
70	PMON45605	451	LCARYYSIASSSKVHPNSVHTCAVVVEYETK---AGRINKGVATNMLRAKEH---AGE--
	human	451	LCARYYSIASSSKVHPNSVHTCAVVVEYETK---AGRINKGVATNMLRAKEH---AGE--
	mouse	451	LCARYYSIASSSKVHPNSVHTCAVVVEYETK---AGRINKGVATNMLRAKEH---AGE--
	PMON45632	448	LCFRYYSIASSSKVHPNSVHTCAVVVEYETK---AGRINKGVATNMLRAKEH---AGE--
	niger	446	LCFRYYSIASSSKVHPNSVHTCAVVVEYETK---AGRINKGVATNMLRAKEH---AGE--
	yeast	436	LCFRYYSIASSSKVHPNSVHTCAVVVEYETK---AGRINKGVATNMLRAKEH---AGE--

5	PMON45605	503	-----NGGF-----ALVPMFVRKSQFRLFFKATTPVIMVGPCTGVAPFI	503	-----NGGF-----ALVPMFVRKSQFRLFFKATTPVIMVGPCTGVAPFI	503	-----NGGF-----ALVPMFVRKSQFRLFFKATTPVIMVGPCTGVAPFI
	human	503	-----NGGF-----ALVPMFVRKSQFRLFFKATTPVIMVGPCTGVAPFI	503	-----NGGF-----ALVPMFVRKSQFRLFFKATTPVIMVGPCTGVAPFI	503	-----NGGF-----ALVPMFVRKSQFRLFFKATTPVIMVGPCTGVAPFI
	mouse	503	-----NGGF-----ALVPMFVRKSQFRLFFKATTPVIMVGPCTGVAPFI	503	-----NGGF-----ALVPMFVRKSQFRLFFKATTPVIMVGPCTGVAPFI	503	-----NGGF-----ALVPMFVRKSQFRLFFKATTPVIMVGPCTGVAPFI
	PMON45632	505	PDPHG-PTYSITCPFNKYDGIHVFVHVHSHNFKLSI-SRPVIMVGPCTGVAPFRGFIQF	505	PDPHG-PTYSITCPFNKYDGIHVFVHVHSHNFKLSI-SRPVIMVGPCTGVAPFRGFIQF	505	PDPHG-PTYSITCPFNKYDGIHVFVHVHSHNFKLSI-SRPVIMVGPCTGVAPFRGFIQF
	niger	503	SRPSR-EDLLHGPFNKYDGIHVFVHVHSHNFKLSI-SRPVIMVGPCTGVAPFRGFIQF	503	SRPSR-EDLLHGPFNKYDGIHVFVHVHSHNFKLSI-SRPVIMVGPCTGVAPFRGFIQF	503	SRPSR-EDLLHGPFNKYDGIHVFVHVHSHNFKLSI-SRPVIMVGPCTGVAPFRGFIQF
10	yeast	495	AETNLPVHYDLNGPFKLEANYKLEFVH/FRSNFRLLSNFSTP/INLPGCTGVAPFPQFIR	495	AETNLPVHYDLNGPFKLEANYKLEFVH/FRSNFRLLSNFSTP/INLPGCTGVAPFPQFIR	495	AETNLPVHYDLNGPFKLEANYKLEFVH/FRSNFRLLSNFSTP/INLPGCTGVAPFPQFIR
	PMON45605	547	R-WLFQ---QKFI---VGETLLVYTCRRPSDELYLHFEELAQFHR-	547	R-WLFQ---QKFI---VGETLLVYTCRRPSDELYLHFEELAQFHR-	547	R-WLFQ---QKFI---VGETLLVYTCRRPSDELYLHFEELAQFHR-
	human	547	R-WLFQ---QKFI---VGETLLVYTCRRPSDELYLHFEELAQFHR-	547	R-WLFQ---QKFI---VGETLLVYTCRRPSDELYLHFEELAQFHR-	547	R-WLFQ---QKFI---VGETLLVYTCRRPSDELYLHFEELAQFHR-
	mouse	547	R-WLFQ---QKFI---VGETLLVYTCRRPSDELYLHFEELAQFHR-	547	R-WLFQ---QKFI---VGETLLVYTCRRPSDELYLHFEELAQFHR-	547	R-WLFQ---QKFI---VGETLLVYTCRRPSDELYLHFEELAQFHR-
	PMON45632	564	R-AALAA---KSEK---VITLLFFTCFKSDEIFLAKDEWKTFOEQADSLKIITAFRRH	564	R-AALAA---KSEK---VITLLFFTCFKSDEIFLAKDEWKTFOEQADSLKIITAFRRH	564	R-AALAA---KSEK---VITLLFFTCFKSDEIFLAKDEWKTFOEQADSLKIITAFRRH
15	niger	562	R-AALAA---KSEK---VITLLFFTCFKSDEIFLAKDEWKTFOEQADSLKIITAFRRH	562	R-AALAA---KSEK---VITLLFFTCFKSDEIFLAKDEWKTFOEQADSLKIITAFRRH	562	R-AALAA---KSEK---VITLLFFTCFKSDEIFLAKDEWKTFOEQADSLKIITAFRRH
	yeast	555	PVAFLESQKKSGNNVSLKHLIFVTSNTL-CFVYQDEWPEVAKKLDSFELGVVHSLP	555	PVAFLESQKKSGNNVSLKHLIFVTSNTL-CFVYQDEWPEVAKKLDSFELGVVHSLP	555	PVAFLESQKKSGNNVSLKHLIFVTSNTL-CFVYQDEWPEVAKKLDSFELGVVHSLP
	PMON45605	599	TSKVTVQHLLPQIRREHLTKLI-EGGAHIYVGGCAPINADVCHTF	599	TSKVTVQHLLPQIRREHLTKLI-EGGAHIYVGGCAPINADVCHTF	599	TSKVTVQHLLPQIRREHLTKLI-EGGAHIYVGGCAPINADVCHTF
	human	599	TSKVTVQHLLPQIRREHLTKLI-EGGAHIYVGGCAPINADVCHTF	599	TSKVTVQHLLPQIRREHLTKLI-EGGAHIYVGGCAPINADVCHTF	599	TSKVTVQHLLPQIRREHLTKLI-EGGAHIYVGGCAPINADVCHTF
	mouse	599	TSKVTVQHLLPQIRREHLTKLI-EGGAHIYVGGCAPINADVCHTF	599	TSKVTVQHLLPQIRREHLTKLI-EGGAHIYVGGCAPINADVCHTF	599	TSKVTVQHLLPQIRREHLTKLI-EGGAHIYVGGCAPINADVCHTF
20	PMON45632	617	SAEYVAVVHR-EBHAEVSDAL-KQKTFYVGGMAHAE-ETVLVGLQILKQRLPAEK	617	SAEYVAVVHR-EBHAEVSDAL-KQKTFYVGGMAHAE-ETVLVGLQILKQRLPAEK	617	SAEYVAVVHR-EBHAEVSDAL-KQKTFYVGGMAHAE-ETVLVGLQILKQRLPAEK
	niger	615	GEQV/VAVVHRLRHS-LVSDAL-KQKTFYVGGMAHAE-ETVLVGLQILKQRLPAEK	615	GEQV/VAVVHRLRHS-LVSDAL-KQKTFYVGGMAHAE-ETVLVGLQILKQRLPAEK	615	GEQV/VAVVHRLRHS-LVSDAL-KQKTFYVGGMAHAE-ETVLVGLQILKQRLPAEK
	yeast	614	NTRV/VV-DKLPDYEDQVFAK-NNISFTVYVGGMAHAE-ETVLVGLQILKQRLPAEK	614	NTRV/VV-DKLPDYEDQVFAK-NNISFTVYVGGMAHAE-ETVLVGLQILKQRLPAEK	614	NTRV/VV-DKLPDYEDQVFAK-NNISFTVYVGGMAHAE-ETVLVGLQILKQRLPAEK
	PMON45605	658	AVDVIFKLNTYGRASLDVAF	658	AVDVIFKLNTYGRASLDVAF	658	AVDVIFKLNTYGRASLDVAF
	human	658	AVDVIFKLNTYGRASLDVAF	658	AVDVIFKLNTYGRASLDVAF	658	AVDVIFKLNTYGRASLDVAF
25	mouse	659	AVDVIFKLNTYGRASLDVAF	659	AVDVIFKLNTYGRASLDVAF	659	AVDVIFKLNTYGRASLDVAF
	PMON45632	676	GBPMVHHRSS-SQDCAAS	676	GBPMVHHRSS-SQDCAAS	676	GBPMVHHRSS-SQDCAAS
	niger	674	GBPMVHHRSS-SQDCAAS	674	GBPMVHHRSS-SQDCAAS	674	GBPMVHHRSS-SQDCAAS
	yeast	673	TELTTHM-KLSGPTQEDV-	673	TELTTHM-KLSGPTQEDV-	673	TELTTHM-KLSGPTQEDV-
30	PMON45605		(SEQ ID NO: 03)		(SEQ ID NO: 03)		(SEQ ID NO: 03)
	human		(SEQ ID NO: 06)		(SEQ ID NO: 06)		(SEQ ID NO: 06)
	mouse		(SEQ ID NO: 39)		(SEQ ID NO: 39)		(SEQ ID NO: 39)
	PMON45632		(SEQ ID NO: 05)		(SEQ ID NO: 05)		(SEQ ID NO: 05)
	niger		(SEQ ID NO: 38)		(SEQ ID NO: 38)		(SEQ ID NO: 38)
35	yeast		(SEQ ID NO: 37)		(SEQ ID NO: 37)		(SEQ ID NO: 37)

Figure 8 – Amino acid homology alignment of *A. ochraceus* oxidoreductase to NADPH cytochrome P450 reductases from *A. niger* and *S. cerevisiae*

5	A. niger	1	NEA LTLDIRVLLVGLVSVAFETETTA/AYTRMPLPA RHH AAPAFPCNTER
	A. ochraceus	1	MAQIDELDLVLLVALLVGSVAETETTA/AYTRMPLPA RHH AAPAFPCNTER
	S. cerevisiae	1	MPFGIINTLFTNLAGLVLLVLLVTRNSIKELMSDDSDITAVSS -----NPDALVV
10	A. niger	60	EEGKNCVIFYGSOTGTAEAYASRLAKESORFGLKTVADLEECVENLLOFFEDRAAF
	A. ochraceus	60	DECKNCVIFYGSOTGTAEAYASRLAKESORFGLKTVADLEECVENLLOFFEDRAAF
	S. cerevisiae	55	TENNYVLLVLTASGTGTARDYAKFSKFLVAKENLVVGLVNVNPFESNDV---VLS
15	A. niger	120	FVLACTAGESEPTENAVEFYTHFEDMAFE--ASAERFLKRTYAFRLNNTVEHYM
	A. ochraceus	120	FVLATYGESEPTENAVEFYTHFEDMAFE--ASAERFLKRTYAFRLNNTVEHYM
	S. cerevisiae	113	IFLSTVTRGFPPCAAFEDFIC-----NABAGA--NRNMFELHSWTFEG
20	A. niger	179	MVFQDAAPCPLEDPISAGEGDDGAGTNEEDFLAFVFMHMALESIDEEFACVTE
	A. ochraceus	180	MVFN/DAALTFFACRIGSAGEGDDGAGTNEEDFLAFVFMHMALESIDEEFACVTE
	S. cerevisiae	163	AARKAKKHLSAENHILKLSEADDAATIDELVMAFDSILEVKEPRLDQRAKETS
25	A. niger	239	VGVTESESLSEEDTYLSEPTSHL-----TPDHYSAHHHFAIAESHEI
	A. ochraceus	240	VFNVTDESLSPEENLSEPTSHL-----TPDHYSAHHHFAIAESHEI
	S. cerevisiae	223	QECY---VNEITDSMSAPFSAHYPSHQLNRNADTQLSEEDLSQAYIAPVSEPI
30	A. niger	290	FTVKDANCLHHEISAGSMLSTGTGDHIAVPTNAAEVDFPLVDFLEHFDVINIK
	A. ochraceus	291	FNVKDRNCLHHEISAGSMLSTGTGDHIAVPTNAAEVDFPLVDFLEHFDVINIK
	S. cerevisiae	280	FSSNDRHCISEPDLSSSHIKSTGDHLAVTISPLEKVEQESHANLDP--STHFDLP
35	A. niger	350	IDVTAKVPIPTFTTYDAAVRYMEACAPVSRQFVATLAAPAMRKABONQWVAG-LE
	A. ochraceus	351	IDVTAKVPIPTFTTYDAAVRYMEACAPVSRQFVATLAAPAMRKABONQWVAG-LE
	S. cerevisiae	338	LIPTFVVFETETICAAIKHLEITETP/SPQLFSSIQEAMNADVBEKILSKETOE
40	A. niger	409	PREGHCPMLCHRALOSITS-KPFSAPVPSLLIEGITHLPRTYSSSSSLVWCKISIF
	A. ochraceus	411	HEKITNRCFNIALALOSITS-KPFTAVPSSLLIEGITHLPRTYSSSSSLVWCKISIF
	S. cerevisiae	398	AVEITSNYCNLIDALKYLSGAKQNVVPMQFLMFSWPCWTPPYTSSESSSSEPTQTHVI
45	A. niger	468	AVVESVRL--EASHNVGVTTNYLLALEKONORSLSRSLDLLHHGPHHYDGIH
	A. ochraceus	470	AVVESVRL--EASHNVGVTTNYLLALEKONCEPSDDEHG--ITSTAPPNYDGIH
	S. cerevisiae	458	SEVFNFPNELELAPPGVSTTTLRNHQLACNVNIAETNLPHFDLNPFKLANYSL
50	A. niger	525	PVHVRSMEKLFSDPSRFIAVGPSTGVAPFRGFQERAAALAAKGE-----VDFVLE
	A. ochraceus	527	PVHVRSMEKLFSDPSRPVIMVGPSTGVAPFRGFQERAAALAAKGE-----VDFVLE
	S. cerevisiae	518	P/HVRSMEKLFSDPSRFIAVGPSTGVAPFRGFQERAAALAAKGE-----VDFVLE
55	A. niger	579	FGCRKSDDEFLVKDEHETTCDDLGCLKIITAFSRH-GPQVTVVIRLFEHSELVSDLLF
	A. ochraceus	581	FGCRKSDDEFLVKDEHETTCDDLGCLKIITAFSRH-SAEVTVVIRLFEHSELVSDLLF
	S. cerevisiae	578	YSENNITDFLTQDEPPAKKLDCSFRVVMHSLPNTKEVYVADKIKLYDQIFEMIN
60	A. niger	638	QKATFYVCGDAAMAREVNLVLGQIIAQQGLPAENGEENVHHHFRDHYQEDWYS
	A. ochraceus	640	QKATFYVCGDAAMAREVNLVLGQIIAQQGLPAENGEENVHHHFRDHYQEDWYS
	S. cerevisiae	637	NCFTFYVCGDAKGVAKGQSTALVGLSLRQKSTTDBATELITMLTSGRPQEDVA-
A. ochraceus, PMON45632 (SEQ ID NO: 05)			
A. niger (SEQ ID NO: 38)			
S. cerevisiae, yeast (SEQ ID NO: 37)			

Figure 9 - Phylogenetic tree showing the relatedness of Aspergillus ochraceus and human oxidoreductase to reductases from A. niger, yeast, and mouse.

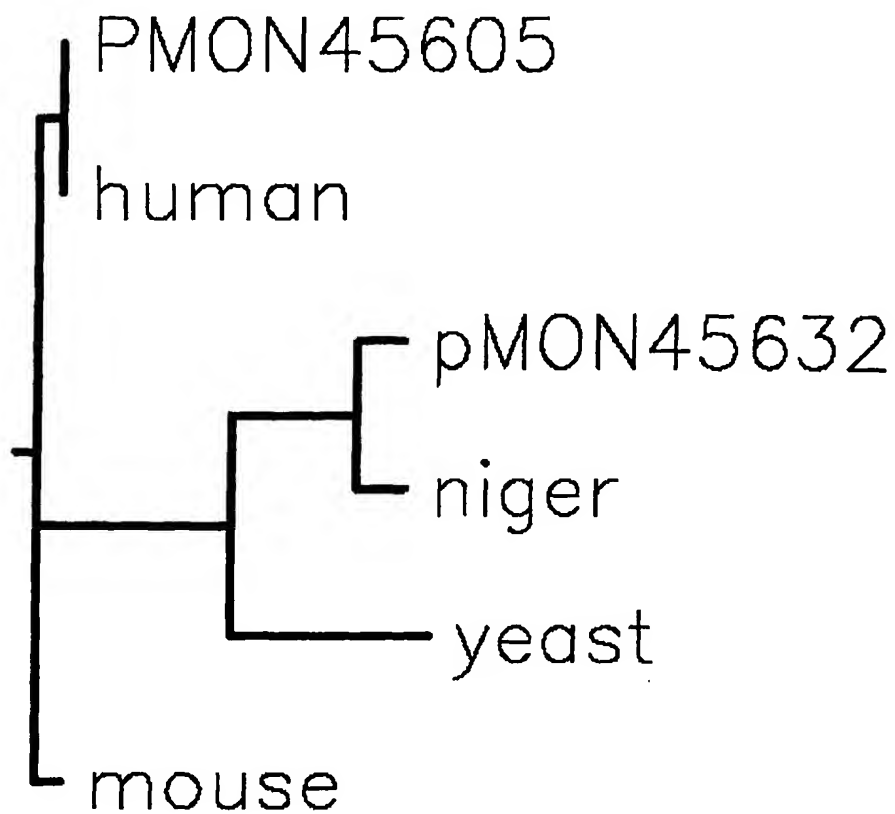


Figure 10 – Percent homology between Aspergillus ochraceus oxidoreductase to reductases from A. niger, yeast, and mouse and human.

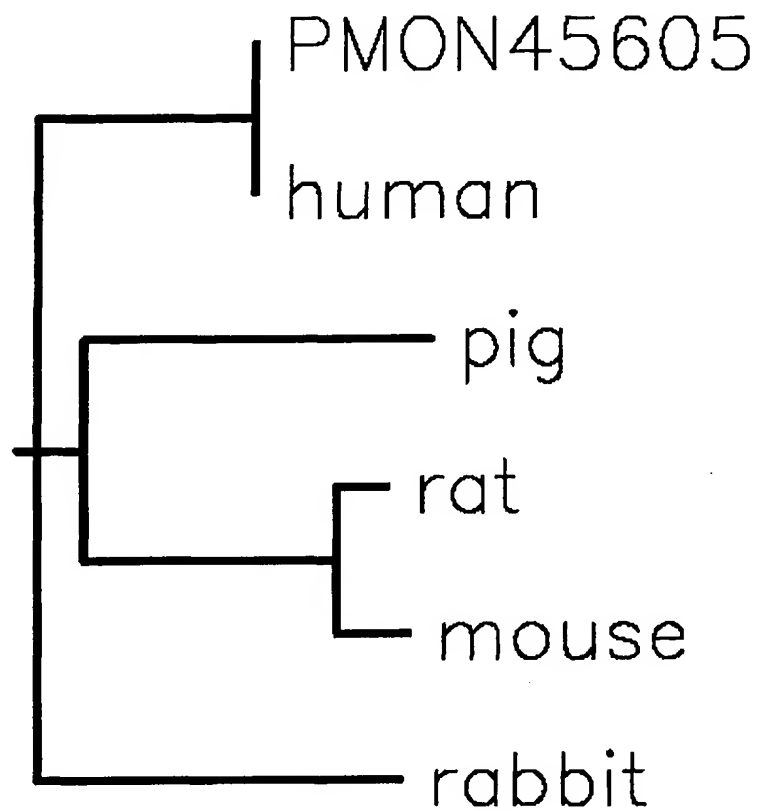
Accession number	organism	% id to A.och oxred
CAA81550	<i>A. niger</i>	84
BAA02936	<i>S. cerevisiae</i>	37
BAA04496	mouse	34
AAB21814	human	33

**Figure 11 – Amino acid homology alignment of human
oxidoreductase with the top 4 hits from SwissProt**

5	PMON45605	1	MGDSH/DTSST	1	SAVAEEVSLFETTER	ICFSLV/LIMTYLFFPYFFET	FEETV	GL
	human	1	MTLSH/DTSST	1	SAVAEEVSLFETTER	ICFSLV/LIMTYLFFPYFFET	FEETV	GL
	rabbit	1	MGDSH/DTSST	1	SAVAEEVSLFETTER	ICFSLV/LIMTYLFFPYFFET	FEETV	GL
	rat	1	MGDSH/DTSST	1	SAVAEEVSLFETTER	ICFSLV/LIMTYLFFPYFFET	FEETV	GL
	mouse	1	MGDSH/DTSST	1	SAVAEEVSLFETTER	ICFSLV/LIMTYLFFPYFFET	FEETV	GL
10	pig	1	MGDSH/DTSST	1	SAVAEEVSLFETTER	ICFSLV/LIMTYLFFPYFFET	FEETV	GL
	PMON45605	61	TSR	61	TSR	TSR	TSR	TSR
	human	61	TSR	61	TSR	TSR	TSR	TSR
	rabbit	61	TSR	61	TSR	TSR	TSR	TSR
	rat	61	APP	61	APP	APP	APP	APP
15	mouse	61	APP	61	APP	APP	APP	APP
	pig	61	TSR	61	TSR	TSR	TSR	TSR
	PMON45605	120	ADLSSLEIDHIAWWSCHATYGRDPTDHCDFYHACETUM	120	ADLSSLEIDHIAWWSCHATYGRDPTDHCDFYHACETUM	ADLSSLEIDHIAWWSCHATYGRDPTDHCDFYHACETUM	ADLSSLEIDHIAWWSCHATYGRDPTDHCDFYHACETUM	ADLSSLEIDHIAWWSCHATYGRDPTDHCDFYHACETUM
	human	120	ADLSSLEIDHIAWWSCHATYGRDPTDHCDFYHACETUM	120	ADLSSLEIDHIAWWSCHATYGRDPTDHCDFYHACETUM	ADLSSLEIDHIAWWSCHATYGRDPTDHCDFYHACETUM	ADLSSLEIDHIAWWSCHATYGRDPTDHCDFYHACETUM	ADLSSLEIDHIAWWSCHATYGRDPTDHCDFYHACETUM
	rabbit	121	ADLSSLEIDHIAWWSCHATYGRDPTDHCDFYHACETUM	121	ADLSSLEIDHIAWWSCHATYGRDPTDHCDFYHACETUM	ADLSSLEIDHIAWWSCHATYGRDPTDHCDFYHACETUM	ADLSSLEIDHIAWWSCHATYGRDPTDHCDFYHACETUM	ADLSSLEIDHIAWWSCHATYGRDPTDHCDFYHACETUM
20	rat	120	ADLSSLEIDHIAWWSCHATYGRDPTDHCDFYHACETUM	120	ADLSSLEIDHIAWWSCHATYGRDPTDHCDFYHACETUM	ADLSSLEIDHIAWWSCHATYGRDPTDHCDFYHACETUM	ADLSSLEIDHIAWWSCHATYGRDPTDHCDFYHACETUM	ADLSSLEIDHIAWWSCHATYGRDPTDHCDFYHACETUM
	mouse	120	ADLSSLEIDHIAWWSCHATYGRDPTDHCDFYHACETUM	120	ADLSSLEIDHIAWWSCHATYGRDPTDHCDFYHACETUM	ADLSSLEIDHIAWWSCHATYGRDPTDHCDFYHACETUM	ADLSSLEIDHIAWWSCHATYGRDPTDHCDFYHACETUM	ADLSSLEIDHIAWWSCHATYGRDPTDHCDFYHACETUM
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	rabbit	181	HFHANGKYVE	181	HFHANGKYVE	HFHANGKYVE	HFHANGKYVE	HFHANGKYVE
	rat	180	HFHANGKYVE	180	HFHANGKYVE	HFHANGKYVE	HFHANGKYVE	HFHANGKYVE
	mouse	180	HFHANGKYVE	180	HFHANGKYVE	HFHANGKYVE	HFHANGKYVE	HFHANGKYVE
	pig	180	HFHANGKYVE	180	HFHANGKYVE	HFHANGKYVE	HFHANGKYVE	HFHANGKYVE
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	rabbit	361	PFPCPTSRYRTALTYLLDITNPRTNVLYELAQYASEPSEQE	361	PFPCPTSRYRTALTYLLDITNPRTNVLYELAQYASEPSEQE	PFPCPTSRYRTALTYLLDITNPRTNVLYELAQYASEPSEQE	PFPCPTSRYRTALTYLLDITNPRTNVLYELAQYASEPSEQE	PFPCPTSRYRTALTYLLDITNPRTNVLYELAQYASEPSEQE
	rat	360	PFPCPTSRYRTALTYLLDITNPRTNVLYELAQYASEPSEQE	360	PFPCPTSRYRTALTYLLDITNPRTNVLYELAQYASEPSEQE	PFPCPTSRYRTALTYLLDITNPRTNVLYELAQYASEPSEQE	PFPCPTSRYRTALTYLLDITNPRTNVLYELAQYASEPSEQE	PFPCPTSRYRTALTYLLDITNPRTNVLYELAQYASEPSEQE
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	mouse	600	SHKVVYVHLLFPDRRLHLLHGGGAHLYVGDPAFRRARDYVTFPDVAELAEHNAV	
	pig	600	POKVVYVHLLFPDRRLHLLHGGGAHLYVGDPAFRRARDYVTFPDVAELAEHNAV	
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	rabbit	661	VDYVKKLTKRYSLDWE	(SEQ ID NO: 53)
	rat	660	VDYVKKLTKRYSLDWE	(SEQ ID NO: 54)
	mouse	660	VDYVKKLTKRYSLDWE	(SEQ ID NO: 55)
	pig	660	VDYVKKLTKRYSLDWE	(SEQ ID NO: 56)
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	human	659	VDYVKKLTKRYSLDWE	(SEQ ID NO: 52)
	rabbit	661	VDYVKKLTKRYSLDWE	(SEQ ID NO: 53)
	rat	660	VDYVKKLTKRYSLDWE	(SEQ ID NO: 54)
	mouse	660	VDYVKKLTKRYSLDWE	(SEQ ID NO: 55)
	pig	660	VDYVKKLTKRYSLDWE	(SEQ ID NO: 56)

**Figure 12 – Phylogenetic tree showing the relatedness of
human oxidoreductase (P16435) with top 4 hits from
SwissProt**

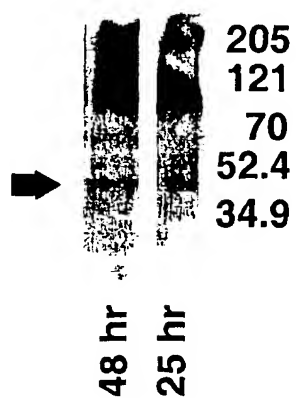


***Figure 13 – Percent homology between human oxidoreductase
and top 4 hits from SwissProt***

Accession number	Species	% id to human oxred
P00388	rat	92
P00389	rabbit	92
P37040	mouse	92
P04175	pig	91

*Figure 14 - Expression of Aspergillus ochraceus 11 alpha
hydroxylase in transfected Sf9 insect cells*

**Expression of 11- α -Hydroxylase in Transfected
Sf9 Cells**



**Figure 15 - Expression of *Aspergillus ochraceus* P450
oxidoreductase in transfected Sf9 insect cells**

**Expression of Fungal P-450 Oxidoreductase
in Transfected Sf9 Cells**

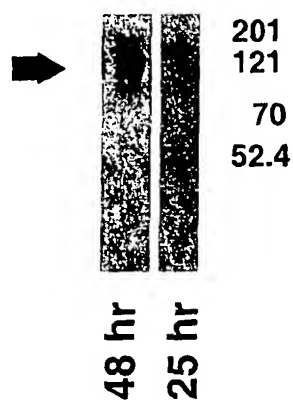
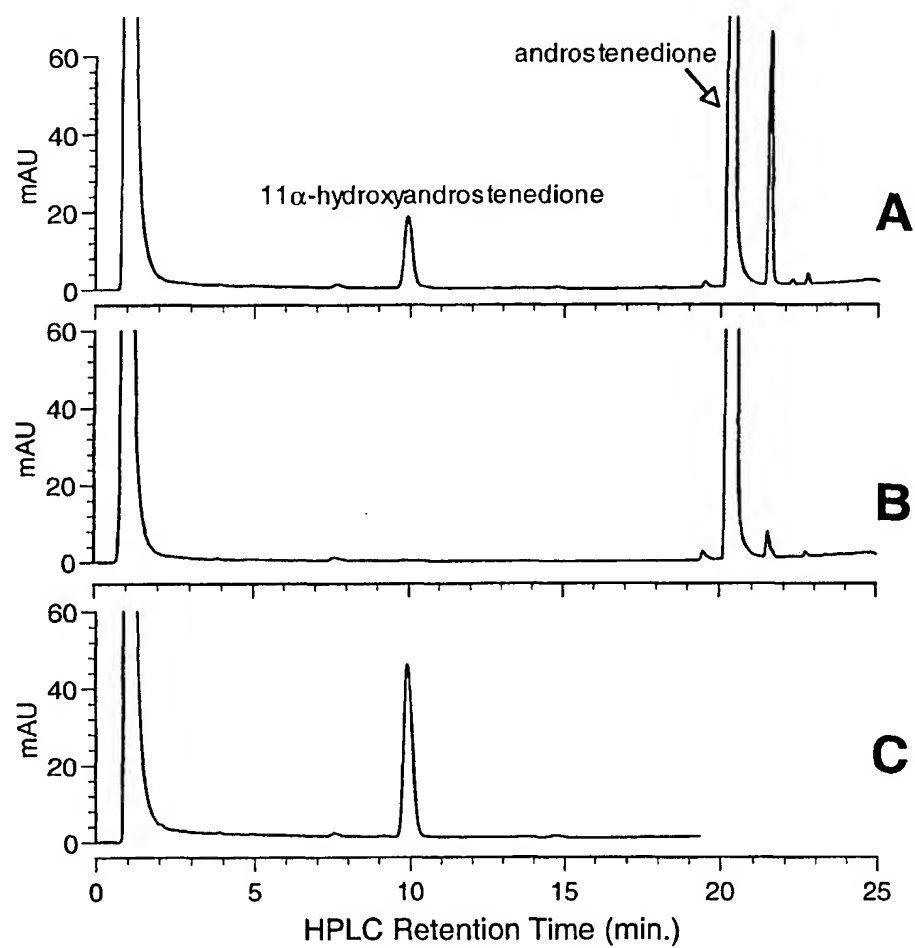


Figure 16 - Conversion of androstenedione to 11 alpha hydroxy androstenedione monitored by HPLC



Sequence Listing

SEQUENCE LISTING

5 <110> Suzanne L. Bolten
 Alan M. Easton
 Leslie C. Engel
 Dean M. Messing
 John S. Ng
 10 Beverly A. Reitz
 Scott A. Vaccaro
 Mark C. Walker
 Ping T. Wang
 Robin A. Weinberg
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45 <211> 20
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Ala Met Asn Gly
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55 <210> 27
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<211> Gibberella fujikuroi CAA75565

<400> 27

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 Ser Leu Ile Leu Gly Thr Cys Ala Val Leu Leu Cys Ile Gln Trp Leu
 35 40 45
 10 Lys Pro Gln Pro Leu Ile Met Val Asn Gly Arg Lys Phe Gly Glu Leu
 50 55 60
 Ser Asn Val Arg Ala Lys Arg Asp Phe Thr Phe Gly Ala Arg Gln Leu
 65 70 75 80
 Leu Glu Lys Gly Leu Lys Met Ser Pro Asp Lys Pro Phe Arg Ile Met
 15 85 90 95
 Gly Asp Val Gly Glu Leu His Ile Leu Pro Pro Lys Tyr Ala Tyr Glu
 100 105 110
 Val Arg Asn Asn Glu Lys Leu Ser Phe Thr Met Ala Ala Phe Lys Trp
 115 120 125
 20 Phe Tyr Ala His Leu Pro Gly Phe Glu Gly Phe Arg Glu Gly Thr Asn
 130 135 140
 Glu Ser His Ile Met Lys Leu Val Ala Arg His Gln Leu Thr His Gln
 145 150 155 160
 Leu Thr Leu Val Thr Gly Ala Val Ser Glu Glu Cys Ala Leu Val Leu
 25 165 170 175
 Lys Asp Val Tyr Thr Asp Ser Pro Glu Trp His Asp Ile Thr Ala Lys
 180 185 190
 Asp Ala Asn Met Lys Leu Met Ala Arg Ile Thr Ser Arg Val Phe Leu
 195 200 205
 30 Gly Lys Glu Met Cys Arg Asn Pro Gln Trp Leu Arg Ile Thr Ser Thr
 210 215 220
 Tyr Ala Val Ile Ala Phe Arg Ala Val Glu Glu Leu Arg Leu Trp Pro
 225 230 235 240
 Ser Trp Leu Arg Pro Val Val Gln Trp Phe Met Pro His Cys Thr Gln
 245 250 255
 35 Ser Arg Ala Leu Val Gln Glu Ala Arg Asp Leu Ile Asn Pro Leu Leu
 260 265 270
 Glu Arg Arg Glu Glu Lys Ala Glu Ala Glu Arg Thr Gly Glu Lys
 275 280 285
 40 Val Thr Tyr Asn Asp Ala Val Glu Trp Leu Asp Asp Leu Ala Arg Glu
 290 295 300
 Lys Gly Val Gly Tyr Asp Pro Ala Cys Ala Gln Leu Ser Leu Ser Val
 305 310 315 320
 Ala Ala Leu His Ser Thr Thr Asp Phe Phe Thr Gln Val Met Phe Asp
 325 330 335
 45 Ile Ala Gln Asn Pro Glu Leu Ile Glu Pro Leu Arg Glu Glu Ile Ile
 340 345 350
 Ala Val Leu Gly Lys Gln Gly Trp Ser Lys Asn Ser Leu Tyr Asn Leu
 355 360 365
 50 Lys Leu Met Asp Ser Val Leu Lys Glu Ser Gln Arg Leu Lys Pro Ile
 370 375 380
 Ala Ile Ala Ser Met Arg Arg Phe Thr Thr His Asn Val Lys Leu Ser
 385 390 395 400
 Asp Gly Val Ile Leu Pro Lys Asn Lys Leu Thr Leu Val Ser Ala His
 405 410 415
 55 Gln His Trp Asp Pro Glu Tyr Tyr Lys Asp Pro Leu Lys Phe Asp Gly
 420 425 430

Tyr Arg Phe Phe Asn Met Arg Arg Glu Pro Gly Lys Glu Ser Lys Ala
 435 440 445
 Gln Leu Val Ser Ala Thr Pro Asp His Met Gly Phe Gly Tyr Gly Leu
 450 455 460
 5 His Ala Cys Pro Gly Arg Phe Phe Ala Ser Glu Glu Ile Lys Ile Ala
 465 470 475 480
 Leu Ser His Ile Leu Leu Lys Tyr Asp Phe Lys Pro Val Glu Gly Ser
 485 490 495
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 25 35 40 45
 Gly Pro Leu Glu Phe Ser Asp Thr Arg Pro Lys Lys Glu Phe Val Tyr
 50 55 60
 Gly Ser Arg Gln Met Leu Ala Asn Trp Phe Lys Ala Asn Pro Asn Lys
 65 70 75 80
 30 Pro Cys Arg Val Ile Ser Asp Phe Gly Glu Ala Ile Val Leu Pro Pro
 85 90 95
 Arg Met Ala Asn Glu Ile Lys Asn Asp Asp Arg Leu Ser Phe Thr Arg
 100 105 110
 Trp Thr Tyr Lys Ala Phe His Gly His Leu Pro Gly Phe Glu Gly Phe
 115 120 125
 35 Gly Glu Ala Ser Arg Glu Ser His Ile Val Gln Glu Val Ile Met Arg
 130 135 140
 Asp Leu Thr Lys Tyr Leu Asn Lys Val Thr Glu Pro Leu Ala Gln Glu
 145 150 155 160
 40 Thr Ser Met Ala Met Glu Ala Asn Leu Pro Lys Ala Ala Asn Gly Glu
 165 170 175
 Trp Ser Thr Ile Asn Leu Arg Ser Lys Ile Leu Pro Ile Val Ala Arg
 180 185 190
 Ile Ser Ser Arg Val Phe Leu Gly Glu Glu Leu Cys Arg Asn Glu Glu
 195 200 205
 45 Trp Leu Lys Val Thr Gln Gln Tyr Thr Ile Asp Gly Phe Gly Ala Ala
 210 215 220
 Glu Asp Leu Arg Leu Trp Pro Ala Ala Leu Arg Pro Ile Val His Trp
 225 230 235 240
 50 Phe Leu Pro Ser Cys Gln Arg Ala Arg Ala Asp Val Arg Val Ala Arg
 245 250 255
 Ser Ile Leu Asp Pro Val Leu Lys Lys Arg Arg Gln Glu Lys Ala Ala
 260 265 270
 Asn Gly Gly Lys Ala Glu His Asp Asp Ala Ile Glu Trp Phe Glu Arg
 275 280 285
 55 Thr Ala Lys Gly Lys Tyr Tyr Asp Pro Ala Val Ala Gln Leu Val Leu
 290 295 300

Ser Leu Val Ala Ile His Thr Thr Ser Asp Leu Thr Cys Gln Val Met
 305 310 315 320
 Thr Asn Leu Met Gln Asn Pro Glu Phe Ile Ala Pro Leu Arg Glu Glu
 325 330 335
 5 Met Ile Gln Val Leu Ser Glu Gly Gly Trp Lys Lys Thr Ser Leu Tyr
 340 345 350
 Asn Met Lys Leu Leu Asp Ser Val Ile Lys Glu Ser Gln Arg Val Lys
 355 360 365
 10 Pro Thr Gly Val Ala Ser Met Arg Arg Tyr Ala Glu Lys Asp Val Thr
 370 375 380
 Leu Ser Asp Gly Thr Phe Ile Pro Lys Gly Gly Phe Val Ala Val Ser
 385 390 395 400
 Ala His Asp Met Trp Asn Ser Glu Val Tyr Glu Gln Ala Glu Lys Trp
 405 410 415
 15 Asp Gly Arg Arg Phe Leu Arg Met Arg Glu Thr Pro Gly Ala Gly Lys
 420 425 430
 Glu Asn Val Ala Gln Leu Val Ser Thr Ala Pro Glu His Leu Gly Phe
 435 440 445
 Gly His Gly Gln His Ala Cys Pro Gly Arg Phe Phe Ala Ala Asn Glu
 450 455 460
 20 Ile Lys Ile Ala Leu Val His Leu Leu Leu Asn Tyr Glu Trp Arg Leu
 465 470 475 480
 Pro Glu Gly Ser Asp Pro Lys Ile Arg Thr Phe Gly Phe Ser Met Gly
 485 490 495
 25 Val Asp Pro Ser Leu Lys Val Glu Tyr Lys Gly Arg Gln Pro Glu Ile
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 Glu Leu

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 <213> Catharanthus roseus CAB56503

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 40 Ile Pro Ile Leu Gly Asn Ala His Gln Leu Ser Gly Gly His Thr His
 35 40 45
 His Ile Leu Arg Asp Leu Ala Lys Lys Tyr Gly Pro Leu Met His Leu
 50 55 60
 Lys Ile Gly Glu Val Ser Thr Ile Val Ala Ser Ser Pro Gln Ile Ala
 65 70 75 80
 45 Glu Glu Ile Phe Arg Thr His Asp Ile Leu Phe Ala Asp Arg Pro Ser
 85 90 95
 Asn Leu Glu Ser Phe Lys Ile Val Ser Tyr Asp Phe Ser Asp Met Val
 100 105 110
 50 Val Ser Pro Tyr Gly Asn Tyr Trp Arg Gln Leu Arg Lys Ile Ser Met
 115 120 125
 Met Glu Leu Leu Ser Gln Lys Ser Val Gln Ser Phe Arg Ser Ile Arg
 130 135 140
 Glu Glu Glu Val Leu Asn Phe Ile Lys Ser Ile Gly Ser Lys Glu Gly
 145 150 155 160
 55 Thr Arg Ile Asn Leu Ser Lys Glu Ile Ser Leu Leu Ile Tyr Gly Ile
 165 170 175

Thr Thr Arg Ala Ala Phe Gly Glu Lys Asn Lys Asn Thr Glu Glu Phe
 180 185 190
 Ile Arg Leu Leu Asp Gln Leu Thr Lys Ala Val Ala Glu Pro Asn Ile
 195 200 205
 5 Ala Asp Met Phe Pro Ser Leu Lys Phe Leu Gln Leu Ile Ser Thr Ser
 210 215 220
 Lys Tyr Lys Ile Glu Lys Ile His Lys Gln Phe Asp Val Ile Val Glu
 225 230 235 240
 Thr Ile Leu Lys Gly His Lys Glu Lys Ile Asn Lys Pro Leu Ser Gln
 245 250 255
 10 Glu Asn Gly Glu Lys Lys Glu Asp Leu Val Asp Val Leu Leu Asn Ile
 260 265 270
 Gln Arg Arg Asn Asp Phe Glu Ala Pro Leu Gly Asp Lys Asn Ile Lys
 275 280 285
 15 Ala Ile Ile Phe Asn Ile Phe Ser Ala Gly Thr Glu Thr Ser Ser Thr
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 Thr Val Asp Trp Ala Met Cys Glu Met Ile Lys Asn Pro Thr Val Met
 305 310 315 320
 Lys Lys Ala Gln Glu Val Arg Lys Val Phe Asn Glu Glu Gly Asn
 325 330 335
 20 Val Asp Glu Thr Lys Leu His Gln Leu Lys Tyr Leu Gln Ala Val Ile
 340 345 350
 Lys Glu Thr Leu Arg Leu His Pro Pro Val Pro Leu Leu Leu Pro Arg
 355 360 365
 25 Glu Cys Arg Glu Gln Cys Lys Ile Lys Gly Tyr Thr Ile Pro Ser Lys
 370 375 380
 Ser Arg Val Ile Val Asn Ala Trp Ala Ile Gly Arg Asp Pro Asn Tyr
 385 390 395 400
 Trp Ile Glu Pro Glu Lys Phe Asn Pro Asp Arg Phe Leu Glu Ser Lys
 405 410 415
 30 Val Asp Phe Lys Gly Asn Ser Phe Glu Tyr Leu Pro Phe Gly Gly Gly
 420 425 430
 Arg Arg Ile Cys Pro Gly Ile Thr Phe Ala Leu Ala Asn Ile Glu Leu
 435 440 445
 35 Pro Leu Ala Gln Leu Leu Phe His Phe Asp Trp Gln Ser Asn Thr Glu
 450 455 460
 Lys Leu Asn Met Lys Glu Ser Arg Gly Val Thr Val Arg Arg Glu Asp
 465 470 475 480
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 485 490 495
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 45 <213> Glycine max AAB94588
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 50 Thr Ser Ile Leu Phe Ile Phe Phe Val Phe Phe Lys Leu Val Gln Arg
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 Ser Asp Ser Lys Thr Ser Ser Thr Cys Lys Leu Pro Pro Gly Pro Arg
 35 40 45
 Thr Leu Pro Leu Ile Gly Asn Ile His Gln Ile Val Gly Ser Leu Pro
 50 55 60
 55 Val His Tyr Tyr Leu Lys Asn Leu Ala Asp Lys Tyr Gly Pro Leu Met
 65 70 75 80

His Leu Lys Leu Gly Glu Val Ser Asn Ile Ile Val Thr Ser Pro Glu
 85 90 95
 Met Ala Gln Glu Ile Met Lys Thr His Asp Leu Asn Phe Ser Asp Arg
 100 105 110
 5 Pro Asp Phe Val Leu Ser Arg Ile Val Ser Tyr Asn Gly Ser Gly Ile
 115 120 125
 Val Phe Ser Gln His Gly Asp Tyr Trp Arg Gln Leu Arg Lys Ile Cys
 130 135 140
 Thr Val Glu Leu Leu Thr Ala Lys Arg Val Gln Ser Phe Arg Ser Ile
 10 145 150 155 160
 Arg Glu Glu Glu Val Ala Glu Leu Val Lys Lys Ile Ala Ala Thr Ala
 165 170 175
 Ser Glu Glu Gly Gly Ser Ile Phe Asn Leu Thr Gln Ser Ile Tyr Ser
 180 185 190
 15 Met Thr Phe Gly Ile Ala Ala Arg Ala Ala Phe Gly Lys Lys Ser Arg
 195 200 205
 Tyr Gln Gln Val Phe Ile Ser Asn Met His Lys Gln Leu Met Leu Leu
 210 215 220
 Gly Gly Phe Ser Val Ala Asp Leu Tyr Pro Ser Ser Arg Val Phe Gln
 20 225 230 235 240
 Met Met Gly Ala Thr Gly Lys Leu Glu Lys Val His Arg Val Thr Asp
 245 250 255
 Arg Val Leu Gln Asp Ile Ile Asp Glu His Lys Asn Arg Asn Arg Ser
 260 265 270
 25 Ser Glu Glu Arg Glu Ala Val Glu Asp Leu Val Asp Val Leu Leu Lys
 275 280 285
 Phe Gln Lys Glu Ser Glu Phe Arg Leu Thr Asp Asp Asn Ile Lys Ala
 290 295 300
 Val Ile Gln Asp Ile Phe Ile Gly Gly Gly Glu Thr Ser Ser Ser Val
 30 305 310 315 320
 Val Glu Trp Gly Met Ser Glu Leu Ile Arg Asn Pro Arg Val Met Glu
 325 330 335
 Glu Ala Gln Ala Glu Val Arg Arg Val Tyr Asp Ser Lys Gly Tyr Val
 340 345 350
 35 Asp Glu Thr Glu Leu His Gln Leu Ile Tyr Leu Lys Ser Ile Ile Lys
 355 360 365
 Glu Thr Met Arg Leu His Pro Pro Val Pro Leu Leu Val Pro Arg Val
 370 375 380
 Ser Arg Glu Arg Cys Gln Ile Asn Gly Tyr Glu Ile Pro Ser Lys Thr
 40 385 390 395 400
 Arg Ile Ile Ile Asn Ala Trp Ala Ile Gly Arg Asn Pro Lys Tyr Trp
 405 410 415
 Gly Glu Thr Glu Ser Phe Lys Pro Glu Arg Phe Leu Asn Ser Ser Ile
 420 425 430
 45 Asp Phe Arg Gly Thr Asp Phe Glu Phe Ile Pro Phe Gly Ala Gly Arg
 435 440 445
 Arg Ile Cys Pro Gly Ile Thr Phe Ala Ile Pro Asn Ile Glu Leu Pro
 450 455 460
 Leu Ala Gln Leu Leu Tyr His Phe Asp Trp Lys Leu Pro Asn Lys Met
 50 465 470 475 480
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 485 490 495
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<212> PRT

<213> Gibberella fujikuroi CAA75566

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 Arg Phe Ser Trp Leu Glu Leu Arg Lys Gly Ser Val Val Pro Leu Ala
 10 35 40 45
 Asn Pro Pro Asp Ser Leu Phe Gly Thr Gly Lys Thr Arg Arg Ser Phe
 50 55 60
 Val Lys Leu Ser Arg Glu Ile Leu Ala Lys Ala Arg Ser Leu Phe Pro
 65 70 75 80
 15 Asn Glu Pro Phe Arg Leu Ile Thr Asp Trp Gly Glu Val Leu Ile Leu
 85 90 95
 Pro Pro Asp Phe Ala Asp Glu Ile Arg Asn Asp Pro Arg Leu Ser Phe
 100 105 110
 Ser Lys Ala Ala Met Gln Asp Asn His Ala Gly Ile Pro Gly Phe Glu
 115 120 125
 20 Thr Val Ala Leu Val Gly Arg Glu Asp Gln Leu Ile Gln Lys Val Ala
 130 135 140
 Arg Lys Gln Leu Thr Lys His Leu Ser Ala Val Ile Glu Pro Leu Ser
 145 150 155 160
 25 Arg Glu Ser Thr Leu Ala Val Ser Leu Asn Phe Gly Glu Thr Thr Glu
 165 170 175
 Trp Arg Ala Ile Arg Leu Lys Pro Ala Ile Leu Asp Ile Ile Ala Arg
 180 185 190
 Ile Ser Ser Arg Ile Tyr Leu Gly Asp Gln Leu Cys Arg Asn Glu Ala
 195 200 205
 30 Trp Leu Lys Ile Thr Lys Thr Tyr Thr Thr Asn Phe Tyr Thr Ala Ser
 210 215 220
 Thr Asn Leu Arg Met Phe Pro Arg Ser Ile Arg Pro Leu Ala His Trp
 225 230 235 240
 35 Phe Leu Pro Glu Cys Arg Lys Leu Arg Gln Glu Arg Lys Asp Ala Ile
 245 250 255
 Gly Ile Ile Thr Pro Leu Ile Glu Arg Arg Arg Glu Leu Arg Arg Ala
 260 265 270
 Ala Ile Ala Ala Gly Gln Pro Leu Pro Val Phe His Asp Ala Ile Asp
 275 280 285
 40 Trp Ser Glu Gln Glu Ala Glu Ala Ala Gly Thr Gly Ala Ser Phe Asp
 290 295 300
 Pro Val Ile Phe Gln Leu Thr Leu Ser Leu Leu Ala Ile His Thr Thr
 305 310 315 320
 45 Tyr Asp Leu Leu Gln Gln Thr Met Ile Asp Leu Gly Arg His Pro Glu
 325 330 335
 Tyr Ile Glu Pro Leu Arg Gln Glu Val Val Gln Leu Leu Arg Glu Glu
 340 345 350
 Gly Trp Lys Lys Thr Thr Leu Phe Lys Met Lys Leu Leu Asp Ser Ala
 355 360 365
 50 Ile Lys Glu Ser Gln Arg Met Lys Pro Gly Ser Ile Val Thr Met Arg
 370 375 380
 Arg Tyr Val Thr Glu Asp Ile Thr Leu Ser Ser Gly Leu Thr Leu Lys
 385 390 395 400
 55 Lys Gly Thr Arg Leu Asn Val Asp Asn Arg Arg Leu Asp Asp Pro Lys
 405 410 415
 Ile Tyr Asp Asn Pro Glu Val Tyr Asn Pro Tyr Arg Phe Tyr Asp Met

420 425 430
 Arg Ser Glu Ala Gly Lys Asp His Gly Ala Gln Leu Val Ser Thr Gly
 435 440 445
 Ser Asn His Met Gly Phe Gly His Gly Gln His Ser Cys Pro Gly Arg
 5 450 455 460
 Phe Phe Ala Ala Asn Glu Ile Lys Val Ala Leu Cys His Ile Leu Val
 465 470 475 480
 Lys Tyr Asp Trp Lys Leu Cys Pro Asp Thr Glu Thr Lys Pro Asp Thr
 485 490 495
 10 Arg Gly Met Ile Ala Lys Ser Ser Pro Val Thr Asp Ile Leu Ile Lys
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 25 Thr Ser Ala Gly Leu Leu Cys Leu Leu Leu Tyr Leu Cys Ser Ser Gly
 35 40 45
 Arg Arg Ser Asp Leu Pro Val Phe Asn Pro Lys Thr Trp Trp Glu Leu
 50 55 60
 Thr Thr Met Arg Ala Lys Arg Asp Phe Asp Ala Asn Ala Pro Ser Trp
 30 65 70 75 80
 Ile Glu Ser Trp Phe Ser Gln Asn Asp Lys Pro Ile Arg Phe Ile Val
 85 90 95
 Asp Ser Gly Tyr Cys Thr Ile Leu Pro Ser Ser Met Ala Asp Glu Phe
 100 105 110
 35 Arg Lys Met Lys Glu Leu Cys Met Tyr Lys Phe Leu Gly Thr Asp Phe
 115 120 125
 His Ser His Leu Pro Gly Phe Asp Gly Phe Lys Glu Val Thr Arg Asp
 130 135 140
 Ala His Leu Ile Thr Lys Val Val Met Asn Gln Phe Gln Thr Gln Ala
 40 145 150 155 160
 Pro Lys Tyr Val Lys Pro Leu Ala Asn Glu Ala Ser Gly Ile Ile Thr
 165 170 175
 Asp Ile Phe Gly Asp Ser Asn Glu Trp His Thr Val Pro Val Tyr Asn
 180 185 190
 45 Gln Cys Leu Asp Leu Val Thr Arg Thr Val Thr Phe Ile Met Val Gly
 195 200 205
 Ser Lys Leu Ala His Asn Glu Glu Trp Leu Asp Ile Ala Lys His His
 210 215 220
 Ala Val Thr Met Ala Ile Gln Ala Arg Gln Leu Arg Leu Trp Pro Val
 50 225 230 235 240
 Ile Leu Arg Pro Leu Val His Trp Leu Glu Pro Gln Gly Ala Lys Leu
 245 250 255
 Arg Ala Gln Val Arg Arg Ala Arg Gln Leu Leu Asp Pro Ile Ile Gln
 260 265 270
 55 Glu Arg Arg Ala Glu Arg Asp Ala Cys Arg Ala Lys Gly Ile Glu Pro
 275 280 285
 Pro Arg Tyr Val Asp Ser Ile Gln Trp Phe Glu Asp Thr Ala Lys Gly

290 295 300
 Lys Trp Tyr Asp Ala Ala Gly Ala Gln Leu Ala Met Asp Phe Ala Gly
 305 310 315 320
 Ile Tyr Gly Thr Ser Asp Leu Leu Ile Gly Gly Leu Val Asp Ile Val
 5 325 330 335
 Arg His Pro His Leu Leu Glu Pro Leu Arg Asp Glu Ile Arg Thr Val
 340 345 350
 Ile Gly Gln Gly Gly Trp Thr Pro Ala Ser Leu Tyr Lys Leu Lys Leu
 355 360 365
 10 Leu Asp Ser Cys Leu Lys Glu Ser Gln Arg Val Lys Pro Val Glu Cys
 370 375 380
 Ala Thr Met Arg Ser Tyr Ala Leu Gln Asp Val Thr Phe Ser Asn Gly
 385 390 395 400
 Thr Phe Ile Pro Lys Gly Glu Leu Val Ala Val Ala Ala Asp Arg Met
 15 405 410 415
 Ser Asn Pro Glu Val Trp Pro Glu Pro Ala Lys Tyr Asp Pro Tyr Arg
 420 425 430
 Tyr Met Arg Leu Arg Glu Asp Pro Ala Lys Ala Phe Ser Ala Gln Leu
 435 440 445
 20 Glu Asn Thr Asn Gly Asp His Ile Gly Phe Gly Trp His Pro Arg Ala
 450 455 460
 Cys Pro Gly Arg Phe Phe Ala Ser Lys Glu Ile Lys Met Met Leu Ala
 465 470 475 480
 Tyr Leu Leu Ile Arg Tyr Asp Trp Lys Val Val Pro Asp Glu Pro Leu
 25 485 490 495
 Gln Tyr Tyr Arg His Ser Phe Ser Val Arg Ile His Pro Thr Thr Lys
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 35 40 45
 Ala Phe Val Gly Leu Pro Leu Cys Arg Asp Glu Gly Trp Leu Gln Ala
 50 55 60
 45 Ser Ile Gly Tyr Thr Val Gln Cys Val Ser Ile Arg Asp Gln Leu Phe
 65 70 75 80
 Thr Trp Ser Pro Val Leu Arg Pro Ile Ile Gly Pro Phe Leu Pro Ser
 85 90 95
 Val Arg Ser Val Arg Arg His Leu Arg Phe Ala Ala Glu Ile Met Ala
 100 105 110
 50 Pro Leu Ile Ser Gln Ala Leu Gln Asp Glu Lys Gln His Arg Ala Asp
 115 120 125
 Thr Leu Leu Ala Asp Gln Thr Glu Gly Arg Gly Thr Phe Ile Ser Trp
 130 135 140
 55 Leu Leu Arg His Leu Pro Glu Glu Leu Arg Thr Pro Glu Gln Val Gly
 145 150 155 160
 Leu Asp Gln Met Leu Val Ser Phe Ala Ala Ile His Thr Thr Thr Met

165 170 175
 Ala Leu Thr Lys Val Val Trp Glu Leu Val Lys Arg Pro Glu Tyr Ile
 180 185 190
 Glu Pro Leu Arg Thr Glu Met Gln Asp Val Phe Gly Pro Asp Ala Val
 5 195 200 205
 Ser Pro Asp Ile Cys Ile Asn Lys Glu Ala Leu Ser Arg Leu His Lys
 210 215 220
 Leu Asp Ser Phe Ile Arg Glu Val Gln Arg Trp Cys Pro Ser Thr Phe
 225 230 235 240
 10 Val Thr Pro Ser Arg Arg Val Met Lys Ser Met Thr Leu Ser Asn Gly
 245 250 255
 Ile Lys Leu Gln Arg Gly Thr Ser Ile Ala Phe Pro Ala His Ala Ile
 260 265 270
 His Met Ser Glu Glu Thr Pro Thr Phe Ser Pro Asp Phe Ser Ser Asp
 15 275 280 285
 Phe Glu Asn Pro Ser Pro Arg Ile Phe Asp Gly Phe Arg Tyr Leu Asn
 290 295 300
 Leu Arg Ser Ile Lys Gly Gln Gly Ser Gln His Gln Ala Ala Thr Thr
 305 310 315 320
 20 Gly Pro Asp Tyr Leu Ile Phe Asn His Gly Lys His Ala Cys Pro Gly
 325 330 335
 Arg Phe Phe Ala Ile Ser Glu Ile Lys Met Ile Leu Ile Glu Leu Leu
 340 345 350
 Ala Lys Tyr Asp Phe Arg Leu Glu Asp Gly Lys Pro Gly Pro Glu Leu
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 35 40 45
 His Val Leu Ser Ser Ser Ser Thr Val Lys Val Pro Val Val Gly Tyr
 50 55 60
 45 Arg Ser Val Phe Glu Pro Thr Trp Leu Leu Arg Leu Arg Phe Val Trp
 65 70 75 80
 Glu Gly Gly Ser Ile Ile Gly Gln Gly Tyr Asn Lys Phe Lys Asp Ser
 85 90 95
 Ile Phe Gln Val Arg Lys Leu Gly Thr Asp Ile Val Ile Ile Pro Pro
 100 105 110
 50 Asn Tyr Ile Asp Glu Val Arg Lys Leu Ser Gln Asp Lys Thr Arg Ser
 115 120 125
 Val Glu Pro Phe Ile Asn Asp Phe Ala Gly Gln Tyr Thr Arg Gly Met
 130 135 140
 55 Val Phe Leu Gln Ser Asp Leu Gln Asn Arg Val Ile Gln Gln Arg Leu
 145 150 155 160
 Thr Pro Lys Leu Val Ser Leu Thr Lys Val Met Lys Glu Glu Leu Asp

165 170 175
 Tyr Ala Leu Thr Lys Glu Met Pro Asp Met Lys Asn Asp Glu Trp Val
 180 185 190
 Glu Val Asp Ile Ser Ser Ile Met Val Arg Leu Ile Ser Arg Ile Ser
 195 200 205
 Ala Arg Val Phe Leu Gly Pro Glu His Cys Arg Asn Gln Glu Trp Leu
 210 215 220
 Thr Thr Thr Ala Glu Tyr Ser Glu Ser Leu Phe Ile Thr Gly Phe Ile
 225 230 235 240
 10 Leu Arg Val Val Pro His Ile Leu Arg Pro Phe Ile Ala Pro Leu Leu
 245 250 255
 Pro Ser Tyr Arg Thr Leu Leu Arg Asn Val Ser Ser Gly Arg Arg Val
 260 265 270
 Ile Gly Asp Ile Ile Arg Ser Gln Gln Gly Asp Gly Asn Glu Asp Ile
 275 280 285
 15 Leu Ser Trp Met Arg Asp Ala Ala Thr Gly Glu Glu Lys Gln Ile Asp
 290 295 300
 Asn Ile Ala Gln Arg Met Leu Ile Leu Ser Leu Ala Ser Ile His Thr
 305 310 315 320
 20 Thr Ala Met Thr Met Thr His Ala Met Tyr Asp Leu Cys Ala Cys Pro
 325 330 335
 Glu Tyr Ile Glu Pro Leu Arg Asp Glu Val Lys Ser Val Val Gly Ala
 340 345 350
 Ser Gly Trp Asp Lys Thr Ala Leu Asn Arg Phe His Lys Leu Asp Ser
 355 360 365
 25 Phe Leu Lys Glu Ser Gln Arg Phe Asn Pro Val Phe Leu Leu Thr Phe
 370 375 380
 Asn Arg Ile Tyr His Gln Ser Met Thr Leu Ser Asp Gly Thr Asn Ile
 385 390 395 400
 30 Pro Ser Gly Thr Arg Ile Ala Val Pro Ser His Ala Met Leu Gln Asp
 405 410 415
 Ser Ala His Val Pro Gly Pro Thr Pro Pro Thr Glu Phe Asp Gly Phe
 420 425 430
 Arg Tyr Ser Lys Ile Arg Ser Asp Ser Asn Tyr Ala Gln Lys Tyr Leu
 435 440 445
 35 Phe Ser Met Thr Asp Ser Ser Asn Met Ala Phe Gly Tyr Gly Lys Tyr
 450 455 460
 Ala Cys Pro Gly Arg Phe Tyr Ala Ser Asn Glu Met Lys Leu Thr Leu
 465 470 475 480
 40 Ala Ile Leu Leu Leu Gln Phe Glu Phe Lys Leu Pro Asp Gly Lys Gly
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 515 520 525

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 Thr Met Leu Asp His Leu Ser Asn Gly Arg Asn Glu His Ile Ala Asp
 50 55 60
 5 Asp Val Glu Leu Gln Leu Leu His Gln Met Thr Leu Ile Ala Val Gly
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 Thr Val Thr Thr Phe Ser Ser Thr Thr Gln Ala Ile Tyr Asp Leu Val
 85 90 95
 Ala His Pro Glu Tyr Ile Thr Ile Leu Arg Glu Glu Val Glu Ser Val
 100 105 110
 10 Pro Arg Asp Pro Asn Gly Asn Phe Thr Lys Asp Ser Thr Val Ala Met
 115 120 125
 Asp Lys Leu Asp Ser Phe Leu Lys Glu Ser Gln Arg Phe Asn Ser Pro
 130 135 140
 15 Asp Leu Ser Met Ser Asn Leu Lys Asn Tyr Lys Leu Cys Glu Ser Leu
 145 150 155 160
 Thr Gly His Ser Asn Leu Pro Thr Arg Thr Ile Ala Asp Met Lys Leu
 165 170 175
 Pro Asp Gly Thr Phe Val Pro Lys Gly Thr Lys Leu Glu Ile Asn Thr
 180 185 190
 20 Cys Ser Ile His Lys Asp His Lys Leu Tyr Glu Asn Pro Glu Gln Phe
 195 200 205
 Asp Gly Leu Arg Phe His Lys Trp Arg Lys Ala Pro Gly Lys Glu Lys
 210 215 220
 Arg Tyr Met Tyr Ser Ser Gly Thr Asp Asp Leu Ser Trp Gly Phe
 225 230 235 240
 25 Gly Arg His Ala Cys Pro Gly Arg Tyr Leu Ser Ala Ile Asn Ile Lys
 245 250 255
 Leu Ile Met Ala Glu Leu Leu Met Asn Tyr Asp Ile Lys Leu Pro Asp
 260 265 270
 30 Gly Leu Ser Arg Pro Lys Asn Ile Glu Phe Glu Val Leu Ala Ser Leu
 275 280 285
 Asn Ala Cys Ala Asn Ala
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 35 <210> 36
 <211> 510
 <212> PRT
 <213> Caenorhabditis elegans CAA91268
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 Phe Ile Tyr Ile Ile Leu Ala Arg Arg Glu Arg Phe Lys Leu Arg Glu
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 45 Lys Ile Gly Leu Ser Gly Pro Glu Pro His Trp Phe Leu Gly Asn Leu
 35 40 45
 Lys Gln Thr Ala Glu Arg Lys Glu Lys Leu Gly Tyr Asp Asp Ala Asn
 50 55 60
 Arg Trp Phe Asn Glu Leu His Glu Gln Tyr Gly Glu Thr Phe Gly Ile
 65 70 75 80
 50 Tyr Tyr Gly Ser Gln Met Asn Ile Val Ile Ser Asn Glu Lys Asp Ile
 85 90 95
 Lys Glu Val Phe Ile Lys Asn Phe Ser Asn Phe Ser Asp Arg Ser Val
 100 105 110
 55 Pro Ser Ile Tyr Glu Ala Asn Gln Leu Thr Ala Ser Leu Leu Met Asn
 115 120 125
 Ser Tyr Ser Ser Gly Trp Lys His Thr Arg Ser Ala Ile Ala Pro Ile

130 135 140
 Phe Ser Thr Gly Lys Met Lys Ala Met Gln Glu Thr Ile Asn Ser Lys
 145 150 155 160
 Val Asp Leu Phe Leu Asp Ile Leu Arg Glu Lys Ala Ser Ser Gly Gln
 5 165 170 175
 Lys Trp Asp Ile Tyr Asp Asp Phe Gln Gly Leu Thr Leu Asp Val Ile
 180 185 190
 Gly Lys Cys Ala Phe Ala Ile Asp Ser Asn Cys Gln Arg Asp Arg Asn
 195 200 205
 10 Asp Val Phe Tyr His Pro Val Thr Val Lys Ile Thr Ile Asn Asn Phe
 210 215 220
 Thr Tyr Phe His Ser Ser Pro Gly Thr Phe His Phe Leu Glu Ser
 225 230 235 240
 Thr Leu Gln Ile His Thr Thr Gly Arg Cys Arg Asn Ser Thr Cys Arg
 15 245 250 255
 Arg Thr Val Lys Cys Val Gly Phe Arg Gln Asp Lys Ala Lys Phe Cys
 260 265 270
 Ser Asp Tyr Glu Arg Arg Arg Gly Gly Glu Gly Ser Asp Ser Val Asp
 275 280 285
 20 Leu Leu Lys Leu Leu Leu Asn Arg Glu Asp Asp Lys Ser Lys Pro Met
 290 295 300
 Thr Lys Gln Glu Val Ile Glu Asn Cys Phe Ala Phe Leu Leu Ala Gly
 305 310 315 320
 Tyr Glu Thr Thr Ser Thr Ala Met Thr Tyr Cys Ser Tyr Leu Leu Ser
 25 325 330 335
 Lys Tyr Pro Asn Val Gln Gln Lys Leu Tyr Glu Glu Ile Met Glu Ala
 340 345 350
 Lys Glu Asn Gly Gly Leu Thr Tyr Asp Ser Ile His Asn Met Lys Tyr
 355 360 365
 30 Leu Asp Cys Val Tyr Lys Glu Thr Leu Arg Phe Tyr Pro Pro His Phe
 370 375 380
 Ser Phe Ile Arg Arg Leu Cys Arg Glu Asp Ile Thr Ile Arg Gly Gln
 385 390 395 400
 Phe Tyr Pro Lys Gly Ala Ile Val Val Cys Leu Pro His Thr Val His
 35 405 410 415
 Arg Asn Pro Glu Asn Trp Asp Ser Pro Glu Glu Phe His Pro Glu Arg
 420 425 430
 Phe Glu Asn Trp Glu Glu Lys Ser Ser Ser Leu Lys Trp Ile Pro Phe
 435 440 445
 40 Gly Val Gly Pro Arg Tyr Cys Val Gly Met Arg Phe Ala Glu Met Glu
 450 455 460
 Phe Lys Thr Thr Ile Val Lys Leu Leu Asp Thr Phe Glu Leu Lys Gln
 465 470 475 480
 Phe Glu Gly Glu Ala Asp Leu Ile Pro Asp Cys Asn Gly Val Ile Met
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 50 <211> 691
 <212> PRT
 <213> yeast P450 reductase

 <400> 37
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 Val Leu Ala Val Leu Leu Tyr Val Lys Arg Asn Ser Ile Lys Glu Leu

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	Leu Met Ser Asp Asp Gly Asp Ile Thr Ala Val Ser Ser Gly Asn Arg		
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5	Asp Ile Ala Gln Val Val Thr Glu Asn Asn Lys Asn Tyr Leu Val Leu		
	50	55	60
	Tyr Ala Ser Gln Thr Gly Thr Ala Glu Asp Tyr Ala Lys Lys Phe Ser		
	65	70	75
	Lys Glu Leu Val Ala Lys Phe Asn Leu Asn Val Met Cys Ala Asp Val		
	85	90	95
10	Glu Asn Tyr Asp Phe Glu Ser Leu Asn Asp Val Pro Val Ile Val Ser		
	100	105	110
	Ile Phe Ile Ser Thr Tyr Gly Glu Gly Asp Phe Pro Asp Gly Ala Val		
	115	120	125
	Asn Phe Glu Asp Phe Ile Cys Asn Ala Glu Ala Gly Ala Leu Ser Asn		
15	130	135	140
	Leu Arg Tyr Asn Met Phe Gly Leu Gly Asn Ser Thr Tyr Glu Phe Phe		
	145	150	155
	Asn Gly Ala Ala Lys Lys Ala Glu Lys His Leu Ser Ala Ala Gly Ala		
	165	170	175
20	Ile Arg Leu Gly Lys Leu Gly Glu Ala Asp Asp Gly Ala Gly Thr Thr		
	180	185	190
	Asp Glu Asp Tyr Met Ala Trp Lys Asp Ser Ile Leu Glu Val Leu Lys		
	195	200	205
	Asp Glu Leu His Leu Asp Glu Gln Glu Ala Lys Phe Thr Ser Gln Phe		
25	210	215	220
	Gln Tyr Thr Val Leu Asn Glu Ile Thr Asp Ser Met Ser Leu Gly Glu		
	225	230	235
	Pro Ser Ala His Tyr Leu Pro Ser His Gln Leu Asn Arg Asn Ala Asp		
	245	250	255
30	Gly Ile Gln Leu Gly Pro Phe Asp Leu Ser Gln Pro Tyr Ile Ala Pro		
	260	265	270
	Ile Val Lys Ser Arg Glu Leu Phe Ser Ser Asn Asp Arg Asn Cys Ile		
	275	280	285
	His Ser Glu Phe Asp Leu Ser Gly Ser Asn Ile Lys Tyr Ser Thr Gly		
35	290	295	300
	Asp His Leu Ala Val Trp Pro Ser Asn Pro Leu Glu Lys Val Glu Gln		
	305	310	315
	Phe Leu Ser Ile Phe Asn Leu Asp Pro Glu Thr Ile Phe Asp Leu Lys		
	325	330	335
40	Pro Leu Asp Pro Thr Val Lys Val Pro Phe Pro Thr Pro Thr Thr Ile		
	340	345	350
	Gly Ala Ala Ile Lys His Tyr Leu Glu Ile Thr Gly Pro Val Ser Arg		
	355	360	365
	Gln Leu Phe Ser Ser Leu Ile Gln Phe Ala Pro Asn Ala Asp Val Lys		
45	370	375	380
	Glu Lys Leu Thr Leu Leu Ser Lys Asp Lys Asp Gln Phe Ala Val Glu		
	385	390	395
	Ile Thr Ser Lys Tyr Phe Asn Ile Ala Asp Ala Leu Lys Tyr Leu Ser		
	405	410	415
50	Asp Gly Ala Lys Trp Asp Asn Val Pro Met Gln Phe Leu Val Glu Ser		
	420	425	430
	Val Pro Gln Met Thr Pro Arg Tyr Tyr Ser Ile Ser Ser Ser Ser Leu		
	435	440	445
	Ser Glu Lys Gln Thr Val His Val Thr Ser Ile Val Glu Asn Phe Pro		
55	450	455	460
	Asn Pro Glu Leu Pro Asp Ala Pro Pro Gly Val Gly Val Thr Thr Asn		
	465	470	475
			480

Leu Leu Arg Asn Ile Gln Leu Ala Gln Asn Asn Val Asn Ile Ala Glu
 485 490 495
 Thr Asn Leu Pro Val His Tyr Asp Leu Asn Gly Pro Arg Lys Leu Phe
 500 505 510
 5 Ala Asn Tyr Lys Leu Pro Val His Val Arg Arg Ser Asn Phe Arg Leu
 515 520 525
 Pro Ser Asn Pro Ser Thr Pro Val Ile Met Ile Gly Pro Gly Thr Gly
 530 535 540
 10 Val Ala Pro Phe Arg Gly Phe Ile Arg Glu Arg Val Ala Phe Leu Glu
 545 550 555 560
 Ser Gln Lys Lys Gly Gly Asn Asn Val Ser Leu Gly Lys His Ile Leu
 565 570 575
 Phe Tyr Gly Ser Arg Asn Thr Asp Asp Phe Leu Tyr Gln Asp Glu Trp
 580 585 590
 15 Pro Glu Tyr Ala Lys Lys Leu Asp Gly Ser Phe Glu Met Val Val Ala
 595 600 605
 His Ser Arg Leu Pro Asn Thr Lys Lys Val Tyr Val Gln Asp Lys Leu
 610 615 620
 20 Lys Asp Tyr Glu Asp Gln Val Phe Glu Met Ile Asn Asn Gly Ala Phe
 625 630 635 640
 Ile Tyr Val Cys Gly Asp Ala Lys Gly Met Ala Lys Gly Val Ser Thr
 645 650 655
 Ala Leu Val Gly Ile Leu Ser Arg Gly Lys Ser Ile Thr Thr Asp Glu
 660 665 670
 25 Ala Thr Glu Leu Ile Lys Met Leu Lys Thr Ser Gly Arg Tyr Gln Glu
 675 680 685
 Asp Val Trp
 690
 30 <210> 38
 <211> 693
 <212> PRT
 <213> Aspergillus niger P450 reductase
 35 <400> 38
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 40 Lys Thr Arg Met Pro Leu Pro Ala Pro Arg Met Asn Gly Ala Ala Lys
 35 40 45
 Ala Gly Lys Thr Arg Asn Ile Ile Glu Lys Met Glu Glu Thr Gly Lys
 50 55 60
 Asn Cys Val Ile Phe Tyr Gly Ser Gln Thr Gly Thr Ala Glu Asp Tyr
 45 65 70 75 80
 Ala Ser Arg Leu Ala Lys Glu Gly Ser Gln Arg Phe Gly Leu Lys Thr
 85 90 95
 Met Val Ala Asp Leu Glu Glu Tyr Asp Tyr Glu Asn Leu Asp Gln Phe
 100 105 110
 50 Pro Glu Asp Lys Val Ala Phe Phe Val Leu Ala Thr Tyr Gly Glu Gly
 115 120 125
 Glu Pro Thr Asp Asn Ala Val Glu Phe Tyr Gln Phe Phe Thr Gly Asp
 130 135 140
 Asp Val Ala Phe Glu Ser Ala Ser Ala Asp Glu Lys Pro Leu Ser Lys
 55 145 150 155 160
 Leu Lys Tyr Val Ala Phe Gly Leu Gly Asn Asn Thr Tyr Glu His Tyr
 165 170 175

Asn Ala Met Val Arg Gln Val Asp Ala Ala Phe Gln Lys Leu Gly Pro
 180 185 190
 Gln Arg Ile Gly Ser Ala Gly Glu Gly Asp Asp Gly Ala Gly Thr Met
 195 200 205
 5 Glu Glu Asp Phe Leu Ala Trp Lys Glu Pro Met Trp Ala Ala Leu Ser
 210 215 220
 Glu Ser Met Asp Leu Glu Glu Arg Glu Ala Val Tyr Glu Pro Val Phe
 225 230 235 240
 10 Cys Val Thr Glu Asn Glu Ser Leu Ser Pro Glu Asp Glu Thr Val Tyr
 245 250 255
 Leu Gly Glu Pro Thr Gln Ser His Leu Gln Gly Thr Pro Lys Gly Pro
 260 265 270
 Tyr Ser Ala His Asn Pro Phe Ile Ala Pro Ile Ala Glu Ser Arg Glu
 275 280 285
 15 Leu Phe Thr Val Lys Asp Arg Asn Cys Leu His Met Glu Ile Ser Ile
 290 295 300
 Ala Gly Ser Asn Leu Ser Tyr Gln Thr Gly Asp His Ile Ala Val Trp
 305 310 315 320
 20 Pro Thr Asn Ala Gly Ala Glu Val Asp Arg Phe Leu Gln Val Phe Gly
 325 330 335
 Leu Glu Gly Lys Arg Asp Ser Val Ile Asn Ile Lys Gly Ile Asp Val
 340 345 350
 Thr Ala Lys Val Pro Ile Pro Thr Pro Thr Thr Tyr Asp Ala Ala Val
 355 360 365
 25 Arg Tyr Tyr Met Glu Val Cys Ala Pro Val Ser Arg Gln Phe Val Ala
 370 375 380
 Thr Leu Ala Ala Phe Ala Pro Met Arg Lys Ala Arg Gln Arg Leu Cys
 385 390 395 400
 Val Trp Val Ala Gln Gly Leu Phe Pro Arg Glu Gly His Gln Pro Met
 405 410 415
 30 Leu Gln His Ala Gln Ala Leu Gln Ser Ile Thr Ser Lys Pro Phe Ser
 420 425 430
 Ala Val Pro Phe Ser Leu Leu Ile Glu Gly Ile Thr Lys Leu Gln Pro
 435 440 445
 35 Arg Tyr Tyr Ser Ile Ser Ser Ser Ser Leu Val Gln Lys Asp Lys Ile
 450 455 460
 Ser Ile Thr Ala Val Val Glu Ser Val Arg Leu Pro Gly Ala Ser His
 465 470 475 480
 Met Val Lys Gly Val Thr Thr Asn Tyr Leu Leu Ala Leu Lys Gln Lys
 485 490 495
 40 Gln Asn Gly Arg Ser Leu Ser Arg Pro Ser Arg Leu Asp Leu Leu His
 500 505 510
 His Gly Pro Arg Asn Lys Tyr Asp Gly Ile His Val Pro Val His Val
 515 520 525
 45 Arg His Ser Asn Phe Lys Leu Pro Ser Asp Pro Ser Arg Pro Ile Ile
 530 535 540
 Met Val Gly Pro Gly Thr Gly Val Ala Pro Phe Arg Gly Phe Ile Gln
 545 550 555 560
 Glu Arg Ala Ala Leu Ala Ala Lys Gly Glu Lys Val Gly Pro Thr Val
 565 570 575
 50 Leu Phe Phe Gly Cys Arg Lys Ser Asp Glu Asp Phe Leu Tyr Lys Asp
 580 585 590
 Glu Trp Lys Thr Tyr Gln Asp Gln Leu Gly Asp Asn Leu Lys Ile Ile
 595 600 605
 55 Thr Ala Phe Ser Arg Glu Gly Pro Gln Lys Val Tyr Val Gln His Arg
 610 615 620
 Leu Arg Glu His Ser Glu Leu Val Ser Asp Leu Leu Lys Gln Lys Ala

625 630 635 640
 Thr Phe Tyr Val Cys Gly Asp Ala Ala Asn Met Ala Arg Glu Val Asn
 645 650 655
 Leu Val Leu Gly Gln Ile Ile Ala Ala Gln Arg Gly Leu Pro Ala Glu
 5 660 665 670
 Lys Gly Glu Glu Met Val Lys His Met Arg Arg Arg Gly Arg Tyr Gln
 675 680 685
 Glu Asp Val Trp Ser
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 <211> 678
 <212> PRT
 <213> mouse
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 Leu Ile Val Gly Val Leu Thr Tyr Trp Phe Ile Phe Lys Lys Lys Lys
 35 40 45
 Glu Glu Ile Pro Glu Phe Ser Lys Ile Gln Thr Thr Ala Pro Pro Val
 50 55 60
 25 65 70 75 80
 Lys Glu Ser Ser Phe Val Glu Lys Met Lys Lys Thr Gly Arg Asn Ile
 Ile Val Phe Tyr Gly Ser Gln Thr Gly Thr Ala Glu Glu Phe Ala Asn
 85 90 95
 Arg Leu Ser Lys Asp Ala His Arg Tyr Gly Met Arg Gly Met Ser Ala
 30 100 105 110
 Asp Pro Glu Glu Tyr Asp Leu Ala Asp Leu Ser Ser Leu Pro Glu Ile
 115 120 125
 Asp Lys Ser Leu Val Val Phe Cys Met Ala Thr Tyr Gly Glu Gly Asp
 130 135 140
 35 145 150 155 160
 Pro Thr Asp Asn Ala Gln Asp Phe Tyr Asp Trp Leu Gln Glu Thr Asp
 Val Asp Leu Thr Gly Val Lys Phe Ala Val Phe Gly Leu Gly Asn Lys
 165 170 175
 Thr Tyr Glu His Phe Asn Ala Met Gly Lys Tyr Val Asp Gln Arg Leu
 40 180 185 190
 Glu Gln Leu Gly Ala Gln Arg Ile Phe Glu Leu Gly Leu Gly Asp Asp
 195 200 205
 Asp Gly Asn Leu Glu Glu Asp Phe Ile Thr Trp Arg Glu Gln Phe Trp
 210 215 220
 45 225 230 235 240
 Pro Ala Val Cys Glu Phe Phe Gly Val Glu Ala Thr Gly Glu Glu Ser
 Ser Ile Arg Gln Tyr Glu Leu Val Val His Glu Asp Met Asp Thr Ala
 245 250 255
 Lys Val Tyr Thr Gly Glu Met Gly Arg Leu Lys Ser Tyr Glu Asn Gln
 50 260 265 270
 Lys Pro Pro Phe Asp Ala Lys Asn Pro Phe Leu Ala Ala Val Thr Thr
 275 280 285
 Asn Arg Lys Leu Asn Gln Gly Thr Glu Arg His Leu Met His Leu Glu
 290 295 300
 55 305 310 315 320
 Leu Asp Ile Ser Asp Ser Lys Ile Arg Tyr Glu Ser Gly Asp His Val
 Ala Val Tyr Pro Ala Asn Asp Ser Thr Leu Val Asn Gln Ile Gly Glu

325 330 335
 Ile Leu Gly Ala Asp Leu Asp Val Ile Met Ser Leu Asn Asn Leu Asp
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 5 Glu Glu Ser Asn Lys Lys His Pro Phe Pro Cys Pro Thr Thr Tyr Arg
 355 360 365
 Thr Ala Leu Thr Tyr Tyr Leu Asp Ile Thr Asn Pro Pro Arg Thr Asn
 370 375 380
 Val Leu Tyr Glu Leu Ala Gln Tyr Ala Ser Glu Pro Ser Glu Gln Glu
 385 390 395 400
 10 His Leu His Lys Met Ala Ser Ser Ser Gly Glu Gly Lys Glu Leu Tyr
 405 410 415
 Leu Ser Trp Val Val Glu Ala Arg Arg His Ile Leu Ala Ile Leu Gln
 420 425 430
 Asp Tyr Pro Ser Leu Arg Pro Pro Ile Asp His Leu Cys Glu Leu Leu
 435 440 445
 15 Pro Arg Leu Gln Ala Arg Tyr Tyr Ser Ile Ala Ser Ser Ser Lys Val
 450 455 460
 His Pro Asn Ser Val His Ile Cys Ala Val Ala Val Glu Tyr Glu Ala
 465 470 475 480
 20 Lys Ser Gly Arg Val Asn Lys Gly Val Ala Thr Ser Trp Leu Arg Thr
 485 490 495
 Lys Glu Pro Ala Gly Glu Asn Gly Arg Arg Ala Leu Val Pro Met Phe
 500 505 510
 Val Arg Lys Ser Gln Phe Arg Leu Pro Phe Lys Pro Thr Thr Pro Val
 515 520 525
 25 Ile Met Val Gly Pro Gly Thr Gly Val Ala Pro Phe Met Gly Phe Ile
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 Gln Glu Arg Ala Trp Leu Arg Glu Gln Gly Lys Glu Val Gly Glu Thr
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 30 Leu Leu Tyr Tyr Gly Cys Arg Arg Ser Asp Glu Asp Tyr Leu Tyr Arg
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 Glu Glu Leu Ala Arg Phe His Lys Asp Gly Ala Leu Thr Gln Leu Asn
 580 585 590
 Val Ala Phe Ser Arg Glu Gln Ala His Lys Val Tyr Val Gln His Leu
 595 600 605
 35 Leu Lys Arg Asp Lys Glu His Leu Trp Lys Leu Ile His Glu Gly Gly
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 Ala His Ile Tyr Val Cys Gly Asp Ala Arg Asn Met Ala Lys Asp Val
 625 630 635 640
 40 Gln Asn Thr Phe Tyr Asp Ile Val Ala Glu Phe Gly Pro Met Glu His
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 Thr Gln Ala Val Asp Tyr Val Lys Lys Leu Met Thr Lys Gly Arg Tyr
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 50 <213> baceriphage M13 reverse primer

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 caggaaacag ctatgac

 55 <210> 41
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<213> bacteriophage T7 promoter primer

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 <210> 42
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 <212> DNA

10 <213> Aspergillus ochraceus Primer 11alphaOH-for

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 <213> Aspergillus ochraceus Primer 11alphaOH-rev

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25 <213> pFastBacI Primer Bacfwd

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30 <210> 45
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 <213> pFastBacI Primer PolyA

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 cctctacaaa tgtggtatg 19

 <210> 46
 <211> 17
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40 <213> Aspergillus ochraceus Primer 45624-for1

 <400> 46
 gagatcaaga ttgcctt 17

45 <210> 47
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 <213> Aspergillus ochraceus Primer 45624-for2

50 <400> 47
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 <210> 48
55 <211> 17
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 <213> Aspergillus ochraceus Primer 45624-rev1

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	<213> human oxidoreductase partial S90469	
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	ttcctcttca gaaagaaaa agaagaagtc cccgagttca ccaaaattca gacattgacc 180	
	tcctctgtca gagagagcag ctttltggaa aagatgaaga aaacggggag gaacatcatc 240	
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	gcccaccgct acgggatgag aggcattgca gcggaccctg aggagtatga cctggccgac 360	
	ctgagcagcc tgccagagat cgacaacgcc ctggtggttt tctgcatggc cactacgggt 420	
	gagggagacc ccaccgacaa tgcccaggac ttctacgact ggctgcagga gacagacgtg 480	
	gatctctctg gggccaagtt cgcggtgttt ggtcttggga acaagacctc cgagcacttc 540	
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	gcacagccca gggcctgcat gggggcaccg ggtccatgc ctctggagcc tctggccctc 2280	
	ggtggtgca cagaagggtc ctttctctct gctgagctgg cccagcccc cccagtgatt 2340	
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	gta	2403
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	<211> 676	
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	<213> human cytochrome P450 reductase, partial AAB21814	

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 Ile Val Gly Leu Leu Thr Tyr Trp Phe Leu Phe Arg Lys Lys Glu
 35 40 45
 Glu Val Pro Glu Phe Thr Lys Ile Gln Thr Leu Thr Ser Ser Val Arg
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 Glu Ser Ser Phe Val Glu Lys Met Lys Lys Thr Gly Arg Asn Ile Ile
 65 70 75 80
 Val Phe Tyr Gly Ser Gln Thr Gly Thr Ala Glu Glu Phe Ala Asn Arg
 85 90 95
 Leu Ser Lys Asp Ala His Arg Tyr Gly Met Arg Gly Met Ser Ala Asp
 100 105 110
 Pro Glu Glu Tyr Asp Leu Ala Asp Leu Ser Ser Leu Pro Glu Ile Asp
 115 120 125
 Asn Ala Leu Val Val Phe Cys Met Ala Thr Tyr Gly Glu Gly Asp Pro
 130 135 140
 Thr Asp Asn Ala Gln Asp Phe Tyr Asp Trp Leu Gln Glu Thr Asp Val
 145 150 155 160
 Asp Leu Ser Gly Val Lys Phe Ala Val Phe Gly Leu Gly Asn Lys Thr
 165 170 175
 Tyr Glu His Phe Asn Ala Met Gly Lys Tyr Val Asp Lys Arg Leu Glu
 180 185 190
 Gln Leu Gly Ala Gln Arg Ile Phe Glu Leu Gly Leu Gly Asp Asp Asp
 195 200 205
 Gly Asn Leu Glu Glu Asp Phe Ile Thr Trp Arg Glu Gln Phe Trp Pro
 210 215 220
 Ala Val Cys Glu His Phe Gly Val Glu Ala Thr Gly Glu Glu Ser Ser
 225 230 235 240
 Ile Arg Gln Tyr Glu Leu Val Val His Thr Asp Ile Asp Ala Ala Lys
 245 250 255
 Val Tyr Met Gly Glu Met Gly Arg Leu Lys Ser Tyr Glu Asn Gln Lys
 260 265 270
 Pro Pro Phe Asp Ala Lys Asn Pro Phe Leu Ala Ala Val Thr Thr Asn
 275 280 285
 Arg Lys Leu Asn Gln Gly Thr Glu Arg His Leu Met His Leu Glu Leu
 290 295 300
 Asp Ile Ser Asp Ser Lys Ile Arg Tyr Glu Ser Gly Asp His Val Ala
 305 310 315 320
 Val Tyr Pro Ala Asn Asp Ser Ala Leu Val Asn Gln Leu Gly Lys Ile
 325 330 335
 Leu Gly Ala Asp Leu Asp Val Val Met Ser Leu Asn Asn Leu Asp Glu
 340 345 350
 Glu Ser Asn Lys Lys His Pro Phe Pro Cys Pro Thr Ser Tyr Arg Thr
 355 360 365
 Ala Leu Thr Tyr Tyr Leu Asp Ile Thr Asn Pro Pro Arg Thr Asn Val
 370 375 380
 Leu Tyr Glu Leu Ala Gln Tyr Ala Ser Glu Pro Ser Glu Gln Glu Leu
 385 390 395 400
 Leu Arg Lys Met Ala Ser Ser Ser Gly Glu Gly Lys Glu Leu Tyr Leu
 405 410 415
 Ser Trp Val Val Glu Ala Arg Arg His Ile Leu Ala Ile Leu Gln Asp
 420 425 430
 Cys Pro Ser Leu Arg Pro Pro Ile Asp His Leu Cys Glu Leu Leu Pro
 435 440 445

Arg Leu Gln Ala Arg Tyr Tyr Ser Ile Ala Ser Ser Ser Lys Val His
 450 455 460
 Pro Asn Ser Val His Ile Cys Ala Val Val Val Glu Tyr Glu Thr Lys
 465 470 475 480
 5 Ala Gly Arg Ile Asn Lys Gly Val Ala Thr Asn Trp Leu Arg Ala Lys
 485 490 495
 Glu Pro Val Gly Glu Asn Gly Gly Arg Ala Leu Val Pro Met Phe Val
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 10 Arg Lys Ser Gln Leu Arg Leu Pro Phe Lys Ala Thr Thr Pro Val Ile
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 Met Val Gly Pro Gly Thr Gly Trp His Pro Phe Ile Gly Phe Ile Gln
 530 535 540
 Glu Arg Ala Trp Leu Arg Gln Gln Gly Lys Glu Val Gly Glu Thr Leu
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 15 Leu Tyr Tyr Gly Cys Arg Arg Ser Asp Glu Asp Tyr Leu Tyr Arg Glu
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 Glu Leu Ala Gln Phe His Arg Asp Gly Ala Leu Thr Gln Leu Asn Val
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 20 Ala Phe Ser Arg Glu Gln Ser His Lys Val Tyr Val Gln His Leu Leu
 595 600 605
 Lys Gln Asp Arg Glu His Leu Trp Lys Leu Ile Glu Gly Gly Ala His
 610 615 620
 Ile Tyr Val Cys Gly Asp Ala Arg Asn Met Ala Arg Asp Val Gln Asn
 625 630 635 640
 25 Thr Phe Tyr Asp Ile Val Ala Glu Leu Gly Ala Met Glu His Ala Gln
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 35 40 45
 Glu Glu Val Pro Glu Phe Thr Lys Ile Gln Thr Leu Thr Ser Ser Val
 45 50 55 60
 Arg Glu Ser Ser Phe Val Glu Lys Met Lys Lys Thr Gly Arg Asn Ile
 65 70 75 80
 Ile Val Phe Tyr Gly Ser Gln Thr Gly Thr Ala Glu Glu Phe Ala Asn
 85 90 95
 50 Arg Leu Ser Lys Asp Ala His Arg Tyr Gly Met Arg Gly Met Ser Ala
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 Asp Pro Glu Glu Tyr Asp Leu Ala Asp Leu Ser Ser Leu Pro Glu Ile
 115 120 125
 Asp Asn Ala Leu Val Val Phe Cys Met Ala Thr Tyr Gly Glu Gly Asp
 130 135 140
 55 Pro Thr Asp Asn Ala Gln Asp Phe Tyr Asp Trp Leu Gln Glu Thr Asp
 145 150 155 160

Val Asp Leu Ser Gly Val Lys Phe Ala Val Phe Gly Leu Gly Asn Lys
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 Thr Tyr Glu His Phe Asn Ala Met Gly Lys Tyr Val Asp Lys Arg Leu
 180 185 190
 5 Glu Gln Leu Gly Ala Gln Arg Ile Phe Glu Leu Gly Leu Gly Asp Asp
 195 200 205
 Asp Gly Asn Leu Glu Glu Asp Phe Ile Thr Trp Arg Glu Gln Phe Trp
 210 215 220
 Pro Ala Val Cys Glu His Phe Gly Val Glu Ala Thr Gly Glu Glu Ser
 225 230 235 240
 10 Ser Ile Arg Gln Tyr Glu Leu Val Val His Thr Asp Ile Asp Ala Ala
 245 250 255
 Lys Val Tyr Met Gly Glu Met Gly Arg Leu Lys Ser Tyr Glu Asn Gln
 260 265 270
 15 Lys Pro Pro Phe Asp Ala Lys Asn Pro Phe Leu Ala Ala Val Thr Thr
 275 280 285
 Asn Arg Lys Leu Asn Gln Gly Thr Glu Arg His Leu Met His Leu Glu
 290 295 300
 Leu Asp Ile Ser Asp Ser Lys Ile Arg Tyr Glu Ser Gly Asp His Val
 305 310 315 320
 20 Ala Val Tyr Pro Ala Asn Asp Ser Ala Leu Val Asn Gln Leu Gly Lys
 325 330 335
 Ile Leu Gly Ala Asp Leu Asp Val Val Met Ser Leu Asn Asn Leu Asp
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 25 Glu Glu Ser Asn Lys Lys His Pro Phe Pro Cys Pro Thr Ser Tyr Arg
 355 360 365
 Thr Ala Leu Thr Tyr Tyr Leu Asp Ile Thr Asn Pro Pro Arg Thr Asn
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 Val Leu Tyr Glu Leu Ala Gln Tyr Ala Ser Glu Pro Ser Glu Gln Glu
 385 390 395 400
 30 Leu Leu Arg Lys Met Ala Ser Ser Ser Gly Glu Gly Lys Glu Leu Tyr
 405 410 415
 Leu Ser Trp Val Val Glu Ala Arg Arg His Ile Leu Ala Ile Leu Gln
 420 425 430
 35 Asp Cys Pro Ser Leu Arg Pro Pro Ile Asp His Leu Cys Glu Leu Leu
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 Pro Arg Leu Gln Ala Arg Tyr Tyr Ser Ile Ala Ser Ser Ser Lys Val
 450 455 460
 His Pro Asn Ser Val His Ile Cys Ala Val Val Val Glu Tyr Glu Thr
 465 470 475 480
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 Lys Glu Pro Ala Gly Glu Asn Gly Gly Arg Ala Leu Val Pro Met Phe
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 45 Val Arg Lys Ser Gln Phe Arg Leu Pro Phe Lys Ala Thr Thr Pro Val
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 Ile Met Val Gly Pro Gly Thr Gly Val Ala Pro Phe Ile Gly Phe Ile
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 Leu Lys Gln Asp Arg Glu His Leu Trp Lys Leu Ile Glu Gly Gly Ala

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 His Ile Tyr Val Cys Gly Asp Ala Arg Asn Met Ala Arg Asp Val Gln
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 Asn Thr Phe Tyr Asp Ile Val Ala Glu Leu Gly Ala Met Glu His Ala
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 Glu Glu Val Pro Glu Phe Thr Lys Ile Gln Thr Leu Thr Ser Ser Val
 50 55 60
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 65 70 75 80
 Ile Val Phe Tyr Gly Ser Gln Thr Gly Thr Ala Glu Glu Phe Ala Asn
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 Asp Pro Glu Glu Tyr Asp Leu Ala Asp Leu Ser Ser Leu Pro Glu Ile
 115 120 125
 Asp Asn Ala Leu Val Val Phe Cys Met Ala Thr Tyr Gly Glu Gly Asp
 130 135 140
 35 Pro Thr Asp Asn Ala Gln Asp Phe Tyr Asp Trp Leu Gln Glu Thr Asp
 145 150 155 160
 Val Asp Leu Ser Gly Val Lys Phe Ala Val Phe Gly Leu Gly Asn Lys
 165 170 175
 Thr Tyr Glu His Phe Asn Ala Met Gly Lys Tyr Val Asp Lys Arg Leu
 40 180 185 190
 Glu Gln Leu Gly Ala Gln Arg Ile Phe Glu Leu Gly Leu Gly Asp Asp
 195 200 205
 Asp Gly Asn Leu Glu Glu Asp Phe Ile Thr Trp Arg Glu Gln Phe Trp
 210 215 220
 45 Pro Ala Val Cys Glu His Phe Gly Val Glu Ala Thr Gly Glu Glu Ser
 225 230 235 240
 Ser Ile Arg Gln Tyr Glu Leu Val Val His Thr Asp Ile Asp Ala Ala
 245 250 255
 Lys Val Tyr Met Gly Glu Met Gly Arg Leu Lys Ser Tyr Glu Asn Gln
 50 260 265 270
 Lys Pro Pro Phe Asp Ala Lys Asn Pro Phe Leu Ala Ala Val Thr Thr
 275 280 285
 Asn Arg Lys Leu Asn Gln Gly Thr Glu Arg His Leu Met His Leu Glu
 290 295 300
 55 Leu Asp Ile Ser Asp Ser Lys Ile Arg Tyr Glu Ser Gly Asp His Val
 305 310 315 320
 Ala Val Tyr Pro Ala Asn Asp Ser Ala Leu Val Asn Gln Leu Gly Lys

325 330 335
 Ile Leu Gly Ala Asp Leu Asp Val Val Met Ser Leu Asn Asn Leu Asp
 340 345 350
 Glu Glu Ser Asn Lys Lys His Pro Phe Pro Cys Pro Thr Ser Tyr Arg
 5 355 360 365
 Thr Ala Leu Thr Tyr Tyr Leu Asp Ile Thr Asn Pro Pro Arg Thr Asn
 370 375 380
 Val Leu Tyr Glu Leu Ala Gln Tyr Ala Ser Glu Pro Ser Glu Gln Glu
 385 390 395 400
 10 Leu Leu Arg Lys Met Ala Ser Ser Ser Gly Glu Gly Lys Glu Leu Tyr
 405 410 415
 Leu Ser Trp Val Val Glu Ala Arg Arg His Ile Leu Ala Ile Leu Gln
 420 425 430
 Asp Cys Pro Ser Leu Arg Pro Pro Ile Asp His Leu Cys Glu Leu Leu
 15 435 440 445
 Pro Arg Leu Gln Ala Arg Tyr Tyr Ser Ile Ala Ser Ser Ser Lys Val
 450 455 460
 His Pro Asn Ser Val His Ile Cys Ala Val Val Val Glu Tyr Glu Thr
 465 470 475 480
 20 Lys Ala Gly Arg Ile Asn Lys Gly Val Ala Thr Asn Trp Leu Arg Ala
 485 490 495
 Lys Glu Pro Ala Gly Glu Asn Gly Gly Arg Ala Leu Val Pro Met Phe
 500 505 510
 Val Arg Lys Ser Gln Phe Arg Leu Pro Phe Lys Ala Thr Thr Pro Val
 25 515 520 525
 Ile Met Val Gly Pro Gly Thr Gly Val Ala Pro Phe Ile Gly Phe Ile
 530 535 540
 Gln Glu Arg Ala Trp Leu Arg Gln Gln Gly Lys Glu Val Gly Glu Thr
 545 550 555 560
 30 Leu Leu Tyr Tyr Gly Cys Arg Arg Ser Asp Glu Asp Tyr Leu Tyr Arg
 565 570 575
 Glu Glu Leu Ala Gln Phe His Arg Asp Gly Ala Leu Thr Gln Leu Asn
 580 585 590
 Val Ala Phe Ser Arg Glu Gln Ser His Lys Val Tyr Val Gln His Leu
 35 595 600 605
 Leu Lys Gln Asp Arg Glu His Leu Trp Lys Leu Ile Glu Gly Gly Ala
 610 615 620
 His Ile Tyr Val Cys Gly Asp Ala Arg Asn Met Ala Arg Asp Val Gln
 625 630 635 640
 40 Asn Thr Phe Tyr Asp Ile Val Ala Glu Leu Gly Ala Met Glu His Ala
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	Ile Val Val Phe Tyr Gly Ser Gln Thr Gly Thr Ala Glu Glu Phe Ala		
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	Asn Arg Leu Ser Lys Asp Ala His Arg Tyr Gly Met Arg Gly Met Ala		
	100	105	110
10	Ala Asp Pro Glu Glu Tyr Asp Leu Ala Asp Leu Ser Ser Leu Pro Glu		
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	Ile Asn Asn Ala Leu Ala Val Phe Cys Met Ala Thr Tyr Gly Glu Gly		
	130	135	140
	Asp Pro Thr Asp Asn Ala Gln Asp Phe Tyr Asp Trp Leu Gln Glu Thr		
15	145	150	155
	Asp Val Asp Leu Ser Gly Val Lys Tyr Ala Val Phe Gly Leu Gly Asn		
	165	170	175
	Lys Thr Tyr Glu His Phe Asn Ala Met Gly Lys Tyr Val Asp Gln Arg		
	180	185	190
20	Leu Glu Gln Leu Gly Ala Gln Arg Ile Phe Glu Leu Gly Met Gly Asp		
	195	200	205
	Asp Asp Ala Asn Leu Glu Glu Asp Phe Ile Thr Trp Arg Glu Gln Phe		
	210	215	220
	Trp Pro Ala Val Cys Glu His Phe Gly Val Glu Ala Thr Gly Glu Glu		
25	225	230	235
	Ser Ser Ile Arg Gln Tyr Glu Leu Val Leu His Thr Asp Ile Asp Val		
	245	250	255
	Ala Lys Val Tyr Gln Gly Glu Met Gly Arg Leu Lys Ser Tyr Glu Asn		
	260	265	270
30	Gln Lys Pro Pro Phe Asp Ala Lys Asn Pro Phe Leu Ala Thr Val Thr		
	275	280	285
	Thr Asn Arg Lys Leu Asn Gln Gly Thr Glu Arg His Leu Met His Leu		
	290	295	300
	Glu Leu Asp Ile Ser Asp Ser Lys Ile Arg Tyr Glu Ser Gly Asp His		
35	305	310	315
	Val Ala Val Tyr Pro Ala Asn Asp Ser Ala Leu Val Asn Gln Leu Gly		
	325	330	335
	Glu Ile Leu Gly Ala Asp Leu Asp Val Val Met Ser Leu Asn Asn Leu		
	340	345	350
40	Asp Glu Glu Ser Asn Lys Lys His Pro Phe Pro Cys Pro Thr Ser Tyr		
	355	360	365
	Arg Thr Ala Leu Thr Tyr Tyr Leu Asp Ile Thr Asn Pro Pro Arg Thr		
	370	375	380
	Asn Val Leu Tyr Glu Leu Ala Gln Tyr Ala Ala Asp Pro Ala Glu Gln		
45	385	390	395
	Glu Gln Leu Arg Lys Met Ala Ser Ser Ser Gly Glu Gly Lys Glu Leu		
	405	410	415
	Tyr Leu Ser Trp Val Val Glu Ala Arg Arg His Ile Leu Ala Ile Leu		
	420	425	430
50	Gln Asp Tyr Pro Ser Leu Arg Pro Pro Ile Asp His Leu Cys Glu Leu		
	435	440	445
	Leu Pro Arg Leu Gln Ala Arg Tyr Tyr Ser Ile Ala Ser Ser Ser Lys		
	450	455	460
	Val His Pro Asn Ser Val His Ile Cys Ala Val Ala Val Glu Tyr Glu		
55	465	470	475
	Thr Lys Ala Gly Arg Leu Asn Lys Gly Val Ala Thr Ser Trp Leu Arg		
	485	490	495

Ala Lys Glu Pro Ala Gly Glu Asn Gly Gly Arg Ala Leu Val Pro Met
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 Phe Val Arg Lys Ser Gln Phe Arg Leu Pro Phe Lys Ala Thr Thr Pro
 515 520 525
 5 Val Ile Met Val Gly Pro Gly Thr Gly Val Ala Pro Phe Ile Gly Phe
 530 535 540
 Ile Gln Glu Arg Ala Trp Leu Arg Gln Gln Gly Lys Glu Val Gly Glu
 545 550 555 560
 10 Thr Leu Leu Tyr Tyr Gly Cys Arg Arg Ala Ala Glu Asp Tyr Leu Tyr
 565 570 575
 Arg Glu Glu Leu Ala Gly Phe Gln Lys Asp Gly Thr Leu Ser Gln Leu
 580 585 590
 Asn Val Ala Phe Ser Arg Glu Gln Ala Gln Lys Val Tyr Val Gln His
 595 600 605
 15 Leu Leu Arg Arg Asp Lys Glu His Leu Trp Arg Leu Ile His Glu Gly
 610 615 620
 Gly Ala His Ile Tyr Val Cys Gly Asp Ala Arg Asn Met Ala Arg Asp
 625 630 635 640
 Val Gln Asn Thr Phe Tyr Asp Ile Val Ala Glu Leu Gly Ala Met Glu
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 20 25 30
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 35 40 45
 Glu Glu Ile Pro Glu Phe Ser Lys Ile Gln Thr Thr Ala Pro Pro Val
 50 55 60
 40 Lys Glu Ser Ser Phe Val Glu Lys Met Lys Lys Thr Gly Arg Asn Ile
 65 70 75 80
 Ile Val Phe Tyr Gly Ser Gln Thr Gly Thr Ala Glu Glu Phe Ala Asn
 85 90 95
 Arg Leu Ser Lys Asp Ala His Arg Tyr Gly Met Arg Gly Met Ser Ala
 100 105 110
 45 Asp Pro Glu Glu Tyr Asp Leu Ala Asp Leu Ser Ser Leu Pro Glu Ile
 115 120 125
 Asp Lys Ser Leu Val Val Phe Cys Met Ala Thr Tyr Gly Glu Gly Asp
 130 135 140
 50 Pro Thr Asp Asn Ala Gln Asp Phe Tyr Asp Trp Leu Gln Glu Thr Asp
 145 150 155 160
 Val Asp Leu Thr Gly Val Lys Phe Ala Val Phe Gly Leu Gly Asn Lys
 165 170 175
 Thr Tyr Glu His Phe Asn Ala Met Gly Lys Tyr Val Asp Gln Arg Leu
 180 185 190
 55 Glu Gln Leu Gly Ala Gln Arg Ile Phe Glu Leu Gly Leu Gly Asp Asp
 195 200 205

Asp Gly Asn Leu Glu Glu Asp Phe Ile Thr Trp Arg Glu Gln Phe Trp
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 Pro Ala Val Cys Glu Phe Phe Gly Val Glu Ala Thr Gly Glu Glu Ser
 225 230 235 240
 5 Ser Ile Arg Gln Tyr Glu Leu Val Val His Glu Asp Met Asp Val Ala
 245 250 255
 Lys Val Tyr Thr Gly Glu Met Gly Arg Leu Lys Ser Tyr Glu Asn Gln
 260 265 270
 10 Lys Pro Pro Phe Asp Ala Lys Asn Pro Phe Leu Ala Ala Val Thr Ala
 275 280 285
 Asn Arg Lys Leu Asn Gln Gly Thr Glu Arg His Leu Met His Leu Glu
 290 295 300
 Leu Asp Ile Ser Asp Ser Lys Ile Arg Tyr Glu Ser Gly Asp His Val
 305 310 315 320
 15 Ala Val Tyr Pro Ala Asn Asp Ser Ala Leu Val Asn Gln Ile Gly Glu
 325 330 335
 Ile Leu Gly Ala Asp Leu Asp Val Ile Met Ser Leu Asn Asn Leu Asp
 340 345 350
 20 Glu Glu Ser Asn Lys Lys His Pro Phe Pro Cys Pro Thr Thr Tyr Arg
 355 360 365
 Thr Ala Leu Thr Tyr Tyr Leu Asp Ile Thr Asn Pro Pro Arg Thr Asn
 370 375 380
 Val Leu Tyr Glu Leu Ala Gln Tyr Ala Ser Glu Pro Ser Glu Gln Glu
 385 390 395 400
 25 His Leu His Lys Met Ala Ser Ser Ser Gly Glu Gly Lys Glu Leu Tyr
 405 410 415
 Leu Ser Trp Val Val Glu Ala Arg Arg His Ile Leu Ala Ile Leu Gln
 420 425 430
 Asp Tyr Pro Ser Leu Arg Pro Pro Ile Asp His Leu Cys Glu Leu Leu
 435 440 445
 30 Pro Arg Leu Gln Ala Arg Tyr Tyr Ser Ile Ala Ser Ser Ser Lys Val
 450 455 460
 His Pro Asn Ser Val His Ile Cys Ala Val Ala Val Glu Tyr Glu Ala
 465 470 475 480
 35 Lys Ser Gly Arg Val Asn Lys Gly Val Ala Thr Ser Trp Leu Arg Ala
 485 490 495
 Lys Glu Pro Ala Gly Glu Asn Gly Gly Arg Ala Leu Val Pro Met Phe
 500 505 510
 Val Arg Lys Ser Gln Phe Arg Leu Pro Phe Lys Ser Thr Thr Pro Val
 515 520 525
 40 Ile Met Val Gly Pro Gly Thr Gly Ile Ala Pro Phe Met Gly Phe Ile
 530 535 540
 Gln Glu Arg Ala Trp Leu Arg Glu Gln Gly Lys Glu Val Gly Glu Thr
 545 550 555 560
 45 Leu Leu Tyr Tyr Gly Cys Arg Arg Ser Asp Glu Asp Tyr Leu Tyr Arg
 565 570 575
 Glu Glu Leu Ala Arg Phe His Lys Asp Gly Ala Leu Thr Gln Leu Asn
 580 585 590
 Val Ala Phe Ser Arg Glu Gln Ala His Lys Val Tyr Val Gln His Leu
 595 600 605
 50 Leu Lys Arg Asp Arg Glu His Leu Trp Lys Leu Ile His Glu Gly Gly
 610 615 620
 Ala His Ile Tyr Val Cys Gly Asp Ala Arg Asn Met Ala Lys Asp Val
 625 630 635 640
 55 Gln Asn Thr Phe Tyr Asp Ile Val Ala Glu Phe Gly Pro Met Glu His
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 Thr Gln Ala Val Asp Tyr Val Lys Lys Leu Met Thr Lys Gly Arg Tyr

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15		Leu Ile Val Gly Val Leu Thr Tyr Trp Phe Ile Phe Lys Lys Lys Lys				
		35 40 45				
		Glu Glu Ile Pro Glu Phe Ser Lys Ile Gln Thr Thr Ala Pro Pro Val				
		50 55 60				
20		Lys Glu Ser Ser Phe Val Glu Lys Met Lys Lys Thr Gly Arg Asn Ile				
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		Ile Val Phe Tyr Gly Ser Gln Thr Gly Thr Ala Glu Glu Phe Ala Asn				
		85 90 95				
		Arg Leu Ser Lys Asp Ala His Arg Tyr Gly Met Arg Gly Met Ser Ala				
		100 105 110				
25		Asp Pro Glu Glu Tyr Asp Leu Ala Asp Leu Ser Ser Leu Pro Glu Ile				
		115 120 125				
		Asp Lys Ser Leu Val Val Phe Cys Met Ala Thr Tyr Gly Glu Gly Asp				
		130 135 140				
30		Pro Thr Asp Asn Ala Gln Asp Phe Tyr Asp Trp Leu Gln Glu Thr Asp				
		145 150 155 160				
		Val Asp Leu Thr Gly Val Lys Phe Ala Val Phe Gly Leu Gly Asn Lys				
		165 170 175				
		Thr Tyr Glu His Phe Asn Ala Met Gly Lys Tyr Val Asp Gln Arg Leu				
		180 185 190				
35		Glu Gln Leu Gly Ala Gln Arg Ile Phe Glu Leu Gly Leu Gly Asp Asp				
		195 200 205				
		Asp Gly Asn Leu Glu Glu Asp Phe Ile Thr Trp Arg Glu Gln Phe Trp				
		210 215 220				
40		Pro Ala Val Cys Glu Phe Phe Gly Val Glu Ala Thr Gly Glu Glu Ser				
		225 230 235 240				
		Ser Ile Arg Gln Tyr Glu Leu Val Val His Glu Asp Met Asp Thr Ala				
		245 250 255				
		Lys Val Tyr Thr Gly Glu Met Gly Arg Leu Lys Ser Tyr Glu Asn Gln				
		260 265 270				
45		Lys Pro Pro Phe Asp Ala Lys Asn Pro Phe Leu Ala Ala Val Thr Thr				
		275 280 285				
		Asn Arg Lys Leu Asn Gln Gly Thr Glu Arg His Leu Met His Leu Glu				
		290 295 300				
50		Leu Asp Ile Ser Asp Ser Lys Ile Arg Tyr Glu Ser Gly Asp His Val				
		305 310 315 320				
		Ala Val Tyr Pro Ala Asn Asp Ser Thr Leu Val Asn Gln Ile Gly Glu				
		325 330 335				
		Ile Leu Gly Ala Asp Leu Asp Val Ile Met Ser Leu Asn Asn Leu Asp				
		340 345 350				
55		Glu Glu Ser Asn Lys Lys His Pro Phe Pro Cys Pro Thr Thr Tyr Arg				
		355 360 365				
		Thr Ala Leu Thr Tyr Tyr Leu Asp Ile Thr Asn Pro Pro Arg Thr Asn				

370 375 380
 Val Leu Tyr Glu Leu Ala Gln Tyr Ala Ser Glu Pro Ser Glu Gln Glu
 385 390 395 400
 His Leu His Lys Met Ala Ser Ser Ser Gly Glu Gly Lys Glu Leu Tyr
 5 405 410 415
 Leu Ser Trp Val Val Glu Ala Arg Arg His Ile Leu Ala Ile Leu Gln
 420 425 430
 Asp Tyr Pro Ser Leu Arg Pro Pro Ile Asp His Leu Cys Glu Leu Leu
 435 440 445
 10 Pro Arg Leu Gln Ala Arg Tyr Tyr Ser Ile Ala Ser Ser Ser Lys Val
 450 455 460
 His Pro Asn Ser Val His Ile Cys Ala Val Ala Val Glu Tyr Glu Ala
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 Lys Ser Gly Arg Val Asn Lys Gly Val Ala Thr Ser Trp Leu Arg Thr
 15 485 490 495
 Lys Glu Pro Ala Gly Glu Asn Gly Arg Arg Ala Leu Val Pro Met Phe
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 Val Arg Lys Ser Gln Phe Arg Leu Pro Phe Lys Pro Thr Thr Pro Val
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 530 535 540
 Gln Glu Arg Ala Trp Leu Arg Glu Gln Gly Lys Glu Val Gly Glu Thr
 545 550 555 560
 Leu Leu Tyr Tyr Gly Cys Arg Arg Ser Asp Glu Asp Tyr Leu Tyr Arg
 25 565 570 575
 Glu Glu Leu Ala Arg Phe His Lys Asp Gly Ala Leu Thr Gln Leu Asn
 580 585 590
 Val Ala Phe Ser Arg Glu Gln Ala His Lys Val Tyr Val Gln His Leu
 595 600 605
 30 Leu Lys Arg Asp Lys Glu His Leu Trp Lys Leu Ile His Glu Gly Gly
 610 615 620
 Ala His Ile Tyr Val Cys Gly Asp Ala Arg Asn Met Ala Lys Asp Val
 625 630 635 640
 Gln Asn Thr Phe Tyr Asp Ile Val Ala Glu Phe Gly Pro Met Glu His
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 35 40 45
 Asp Glu Val Pro Glu Phe Ser Lys Ile Glu Thr Thr Thr Ser Ser Val
 50 55 60
 55 Lys Asp Ser Ser Phe Val Glu Lys Met Lys Lys Thr Gly Arg Asn Ile
 65 70 75 80
 Ile Val Phe Tyr Gly Ser Gln Thr Gly Thr Ala Glu Glu Phe Ala Asn

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	Arg Leu Ser Lys Asp Ala His	Arg Tyr Gly Met Arg Gly Met Ala Ala	
	100	105	110
5	Asp Pro Glu Glu Tyr Asp Leu Ser	Asp Leu Ser Ser Leu Pro Glu Ile	
	115	120	125
	Glu Asn Ala Leu Ala Val Phe Cys Met Ala Thr Tyr Gly Glu Gly Asp		
	130	135	140
	Pro Thr Asp Asn Ala Gln Asp Phe Tyr Asp Trp Leu Gln Glu Ala Asp		
	145	150	155
10	Val Asp Leu Thr Gly Val Lys Tyr Ala Val Phe Gly Leu Gly Asn Lys		
	165	170	175
	Thr Tyr Glu His Phe Asn Ala Met Gly Lys Tyr Val Asp Lys Arg Leu		
	180	185	190
	Glu Gln Leu Gly Ala Gln Arg Ile Phe Asp Leu Gly Leu Gly Asp Asp		
15	195	200	205
	Asp Gly Asn Leu Glu Glu Asp Phe Ile Thr Trp Arg Glu Gln Phe Trp		
	210	215	220
	Pro Ala Val Cys Glu His Phe Gly Val Glu Ala Thr Gly Glu Glu Ser		
	225	230	235
20	Ser Ile Arg Gln Tyr Glu Leu Val Val His Thr Asp Met Asp Thr Ala		
	245	250	255
	Val Val Tyr Thr Gly Glu Met Gly Arg Leu Lys Ser Tyr Glu Asn Gln		
	260	265	270
	Lys Pro Pro Phe Asp Ala Lys Asn Pro Phe Leu Ala Val Val Thr Thr		
25	275	280	285
	Asn Arg Lys Leu Asn Gln Gly Thr Glu Arg His Leu Met His Leu Glu		
	290	295	300
	Leu Asp Ile Ser Asp Ser Lys Ile Arg Tyr Glu Ser Gly Asp His Val		
	305	310	315
30	Ala Val Tyr Pro Ala Asn Asp Ser Ala Leu Val Asn Gln Leu Gly Glu		
	325	330	335
	Ile Leu Gly Thr Asp Leu Asp Ile Val Met Ser Leu Asn Asn Leu Asp		
	340	345	350
	Glu Glu Ser Asn Lys Arg His Pro Phe Pro Cys Pro Thr Thr Tyr Arg		
35	355	360	365
	Thr Ala Leu Thr Tyr Tyr Leu Asp Ile Thr Asn Pro Pro Arg Thr Asn		
	370	375	380
	Val Leu Tyr Glu Leu Ala Gln Tyr Ala Ser Glu Pro Ser Glu Gln Glu		
	385	390	395
40	Gln Leu Arg Lys Met Ala Ser Ser Ser Gly Glu Gly Lys Glu Leu Tyr		
	405	410	415
	Leu Ser Trp Val Val Glu Ala Arg Arg His Ile Leu Ala Ile Leu Gln		
	420	425	430
	Asp Tyr Pro Ser Leu Arg Pro Pro Ile Asp His Leu Cys Glu Arg Leu		
45	435	440	445
	Pro Arg Leu Gln Ala Arg Tyr Tyr Ser Ile Ala Ser Ser Ser Lys Val		
	450	455	460
	His Pro Asn Ser Val His Ile Cys Ala Val Val Val Glu Tyr Glu Thr		
	465	470	475
50	Lys Ser Gly Arg Val Asn Lys Gly Val Ala Thr Ser Trp Leu Arg Ala		
	485	490	495
	Lys Glu Pro Ala Gly Glu Asn Gly Arg Arg Ala Leu Val Pro Met Phe		
	500	505	510
	Val Arg Lys Ser Gln Phe Arg Leu Pro Phe Lys Ala Thr Thr Pro Val		
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	Ile Met Val Gly Pro Gly Thr Gly Val Ala Pro Phe Ile Gly Phe Ile		
	530	535	540

Gln Glu Arg Ala Trp Leu Gln Glu Gln Gly Lys Glu Val Gly Glu Thr
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 Leu Leu Tyr Tyr Gly Cys Arg Arg Ser Asp Glu Asp Tyr Leu Tyr Arg
 565 570 575
 5 Glu Glu Leu Ala Gln Phe His Ala Lys Gly Ala Leu Thr Arg Leu Ser
 580 585 590
 Val Ala Phe Ser Arg Glu Gln Pro Gln Lys Val Tyr Val Gln His Leu
 595 600 605
 10 Leu Lys Arg Asp Lys Glu His Leu Trp Lys Leu Ile His Asp Gly Gly
 610 615 620
 Ala His Ile Tyr Ile Cys Gly Asp Ala Arg Asn Met Ala Arg Asp Val
 625 630 635 640
 Gln Asn Thr Phe Cys Asp Ile Val Ala Glu Gln Gly Pro Met Glu His
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 Ser Leu Asp Val Trp Ser
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 55 <211> 12
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 <223> Bottom Strand of a SalI adapter, the first base is phosphorylated

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	Asn Cys Val Ile Phe Tyr Gly Ser Gln Thr Gly Thr Ala Glu Asp Tyr		
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	Ala Ser Arg Leu Ala Lys Glu Gly Ser Gln Arg Phe Gly Leu Lys Thr		
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10	Met Val Ala Asp Leu Glu Glu Tyr Asp Tyr Glu Asn Leu Asp Gln Phe		
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	Pro Glu Asp Lys Val Ala Phe Phe Val Leu Ala Thr Tyr Gly Glu Gly		
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15	Glu Pro Thr Asp Asn Ala Val Glu Phe Tyr Gln Phe Phe Thr Gly Asp		
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	Asp Val Ala Phe Glu Ser Ala Ser Ala Asp Glu Lys Pro Leu Ser Lys		
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	Leu Lys Tyr Val Ala Phe Gly Leu Gly Asn Asn Thr Tyr Glu His Tyr		
	165	170	175
20	Asn Ala Met Val Arg Gln Val Asp Ala Ala Phe Gln Lys Leu Gly Pro		
	180	185	190
	Gln Arg Ile Gly Ser Ala Gly Glu Gly Asp Asp Gly Ala Gly Thr Met		
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25	Glu Glu Asp Phe Leu Ala Trp Lys Glu Pro Met Trp Ala Ala Leu Ser		
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	Glu Ser Met Asp Leu Glu Glu Arg Glu Ala Val Tyr Glu Pro Val Phe		
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	Cys Val Thr Glu Asn Glu Ser Leu Ser Pro Glu Asp Glu Thr Val Tyr		
	245	250	255
30	Leu Gly Glu Pro Thr Gln Ser His Leu Gln Gly Thr Pro Lys Gly Pro		
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	Tyr Ser Ala His Asn Pro Phe Ile Ala Pro Ile Ala Glu Ser Arg Glu		
	275	280	285
35	Leu Phe Thr Val Lys Asp Arg Asn Cys Leu His Met Glu Ile Ser Ile		
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	Ala Gly Ser Asn Leu Ser Tyr Gln Thr Gly Asp His Ile Ala Val Trp		
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	Pro Thr Asn Ala Gly Ala Glu Val Asp Arg Phe Leu Gln Val Phe Gly		
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45	Arg Tyr Tyr Met Glu Val Cys Ala Pro Val Ser Arg Gln Phe Val Ala		
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	Thr Leu Ala Ala Phe Ala Pro Met Arg Lys Ala Arg Gln Arg Leu Cys		
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	Val Trp Val Ala Gln Gly Leu Phe Pro Arg Glu Gly His Gln Pro Met		
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	Ser Ile Thr Ala Val Val Glu Ser Val Arg Leu Pro Gly Ala Ser His		
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			480

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 Leu Phe Phe Gly Cys Arg Lys Ser Asp Glu Asp Phe Leu Tyr Lys Asp
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